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FORM PTO-1390 (REV 10-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C.371

ATTORNEY'S DOCKET NUMBER: **GIN-6715CPUS**

U.S. APPLICATION NO. (If known, see 37 CFR 1.5): **09/674235**

INTERNATIONAL APPLICATION	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/JP99/02226	**27 April 1999 (27.04.99)**	**28 April 1998 (28.04.98)**

TITLE OF INVENTION

HUMAN PROTEINS HAVING TRANSMEMBRANE DOMAINS AND DNAS ENCODING THESE PROTEINS

APPLICANT(S) FOR DO/EO/US

Seishi KATO and Tomoko KIMURA

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C.371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371 (f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371 (b) and PCT Articles 22 and 39(1).
4. ☐ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)) **(unexecuted) (4 sheets);**
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included
13. ☐ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information: **Transmittal Letter (2 sheets in duplicate); PCT Request (4 sheets); PCT Notification of Receipt of Record Copy (1 sheet); PCT Notification Concerning Submission or Transmittal of Priority Document (1 sheet); PCT Notice Informing the Applicant of the Communication of the International Application to the Designated Offices (1 sheet); PCT International Published Application (WO 99/55862) (without International Search Report) (116 sheets); Cover Sheet of PCT International Published Application (WO 99/55862) (with International Search Report attached); (7 sheets); PCT International Preliminary Examination Report (5 sheets); Sequence Listing (25 sheets) along with Transmittal Letter and Diskette for Sequence Listing (1 sheet); Check (#039743) ($1230); Certificate of Express Mailing (1 sheet); and Return Postcard.**

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NO.
09/674235	PCT/JP99/02226	GIN-6715CPUS

17. ☒ The following fees are submitted:

CALCULATIONS PTO USE ONLY	

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) .(a/o January 1, 2000):

Search Report has been prepared by the EPO or JPO.....$970

International preliminary examination fee paid to USPTO (37 CFR 1.482).....$840

No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)).....$690

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....$670

International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4).....$96

ENTER APPROPRIATE BASIC FEE AMOUNT =				**$970**	
Surcharge of **$130.00** for furnishing the oath or declaration later than 20 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				$--	
CLAIMS	**NUMBER FILED**		**NUMBER EXTRA**	**RATE**	
Total claims	**10**	-20 =	**0**	X $18.00	**$0**
Independent claims	**2**	-3 =	**0**	X $78.00	**$0**
MULTIPLE DEPENDENT CLAIM(S) (if applicable)				+ 260.00	**$260**
TOTAL OF ABOVE CALCULATIONS =					**$1230**
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28)					$--
SUBTOTAL =					**$1230**
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +					$--
TOTAL NATIONAL FEE =					**$1230**
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +					$
TOTAL FEES ENCLOSED =					**$1230**
				Amount to be: refunded	$
				charged	$

a. ☒ A check **(# 039743)** in the amount of **$1230** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **12-0080**. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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SIGNATURE

Peter C. Lauro
NAME

32,360
REGISTRATION NUMBER

DESCRIPTION

HUMAN PROTEINS HAVING TRANSMEMBRANE DOMAINS AND DNAs ENCODING THESE PROTEINS

TECHNICAL FIELD

The present invention relates to human proteins having transmembrane domains, cDNAs coding for these proteins, and expression vectors of said cDNAs as well as eucaryotic cells expressing said cDNAs. The proteins of the present invention can be employed as pharmaceuticals or as antigens for preparing antibodies against said proteins. The human cDNAs of the present invention can be utilized as probes for the gene diagnosis and gene sources for the gene therapy. Furthermore, the cDNAs can be utilized as gene sources for large-scale production of the proteins encoded by said cDNAs. Cells, wherein these membrane protein genes are introduced and membrane proteins are expressed in large amounts, can be utilized for detection of the corresponding ligands, screening of novel low-molecular pharmaceuticals, and so on.

BACKGROUND ART

Membrane proteins play important roles, as signal receptors, ion channels, transporters, etc. in the material transportation and the information transmission which are mediated by the cell membrane. Examples thereof include receptors for a variety of cytokines, ion channels for the sodium ion, the potassium ion, the chloride ion, etc., transporters for saccharides and amino acids, and so on, where the genes of many of them have been cloned already.

It has been clarified that abnormalities of these membrane proteins are associated with a number of hitherto-

cryptogenic diseases. For instance, a gene of a membrane protein having twelve transmembrane domains was identified as the gene responsible for cystic fibrosis [Rommens, J. M. et al., Science 245: 1059-1065 (1989)]. In addition, it has been clarified that several membrane proteins act as receptors when a virus infects the cells. For instance, HIV-1 is revealed to infect into the cells through mediation of a membrane protein fusin having a membrane protein on the T-cell membrane, a CD-4 antigen, and seven transmembrane domains [Feng, Y. et al., Science 272: 872-877 (1996)]. Therefore, discovery of a new membrane protein is anticipated to lead to elucidation of the causes of many diseases, so that isolation of a new gene coding for the membrane protein has been desired.

Heretofore, owing to difficulty in the purification, many membrane proteins have been isolated by an approach from the gene side. A general method is the so-called expression cloning which comprises transfection of a cDNA library in eucaryotic cells to express cDNAs and then detection of the cells expressing the target membrane protein on the membrane by an immunological technique using an antibody or a physiological technique on the change in the membrane permeability. However, this method is applicable only to cloning of a gene of a membrane protein with a known function.

In general, membrane proteins possess hydrophobic transmembrane domains inside the proteins, wherein, after synthesis thereof in the ribosome, these domains remain in the phospholipid membrane to be trapped in the membrane. Accordingly, the evidence of the cDNA for encoding the membrane protein is provided by determination of the whole base sequence of a full-length cDNA followed by detection of highly hydrophobic transmembrane domains in the amino acid sequence of the protein encoded by said cDNA.

DISCLOSURE OF INVENTION

The object of the present invention is to provide novel human proteins having transmembrane domains, DNAs coding for said proteins, and expression vectors of said DNAs as well as transformation eucaryotic cells that are capable of expressing said DNAs.

As the result of intensive studies, the present inventors have been successful in cloning of cDNAs coding for proteins having transmembrane domains from the human full-length cDNA bank, thereby completing the present invention. In other words, the present invention provides human proteins having transmembrane domains, namely proteins containing any of the amino acid sequences represented by Sequence Nos. 1 to 9. Moreover, the present invention provides DNAs coding for the above-mentioned proteins, exemplified by cDNAs containing any of the base sequences represented by Sequence Nos. 10 to 19, 21, 23, 25, 27, 29, 31, 33 and 35, as well as expression vectors that are capable of expressing any of said DNAs by in vitro translation or in eucaryotic cells and transformation eucaryotic cells that are capable of expressing said DNAs and of producing the above-mentioned proteins.

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP02000.

Fig. 2: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP02061.

Fig. 3: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by

clone HP02163.

Fig. 4: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP02219.

Fig. 5: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP02256.

Fig. 6: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10390.

Fig. 7: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10474.

Fig. 8: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10527.

Fig. 9: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10528.

BEST MODE FOR CARRYING OUT THE INVENTION

The proteins of the present invention can be obtained, for example, by a method for isolation from human organs, cell lines, etc., a method for preparation of peptides by the chemical synthesis, or a method for production with the recombinant DNA technology using the DNAs coding for the transmembrane domains of the present invention, wherein the method for obtainment by the recombinant DNA technology is employed preferably. For instance, in vitro expression of the proteins can be achieved by preparation of an RNA by in vitro transcription from a vector having one of cDNAs of the present invention, followed by in vitro translation using this RNA as a

template. Also, recombination of the translation region into a suitable expression vector by the method known in the art leads to expression of a large amount of the encoded protein by using prokaryotic cells such as *Escherichia coli, Bacillus subtilis*, etc., and eucaryotic cells such as yeasts, insect cells, mammalian cells, etc.

In the case in which one of the proteins of the present invention is produced by expressing the DNA by in vitro translation, the protein of the present invention can be produced in vitro, when the translation region of said cDNA is subjected to recombination to a vector having an RNA polymerase promoter, followed by addition to an in vitro translation system such as a rabbit riticulocyte lysate or a wheat germ extract, containing an RNA polymerase corresponding to the promoter. RNA polymerase inhibitors are exemplified by T7, T3, SP6, and the like. The vectors containing these RNA polymerase inhibitors are exemplified by pKA1, pCDM8, pT3/7 18, pT7/3 19, pBluescript II, and so on. Furthermore, a membrane protein of the present invention can be expressed as the form incorporated in the microsome membrane, when a dog pancreas microsome or the like is added into the reaction system.

In the case in which a protein of the present invention is produced by expressing the DNA using a microorganism such as *Escherichia coli* etc., a recombinant expression vector bearing the translation region in the cDNA of the present invention is constructed in an expression vector having an origin, a promoter, a ribosome-binding site, a cDNA-cloning site, a terminator etc., which can be replicated in the microorganism, and, after transformation of the host cells with said expression vector, the thus-obtained transformant is incubated, whereby the protein encoded by said cDNA can be produced on a large scale in the microorganism. In this case, a protein

fragment containing an optional region can be obtained by carrying out the expression with inserting an initiation codon and a termination codon in front of and behind an optional translation region. Alternatively, a fusion protein with another protein can be expressed. Only a protein portion coding for said cDNA can be obtained by cleavage of said fusion protein with a suitable protease. The expression vector for *Escherichia coli* is exemplified by the pUC system, pBluescript II, the pET expression system, the pGEX expression system, and so on.

In the case in which one of the proteins of the present invention is produced by expressing the DNA in eucaryotic cells, the protein of the present invention can be produced as a transmembrane protein on the cell-membrane surface, when the translation region of said cDNA is subjected to recombination to an expression vector for eucaryotic cells that has a promoter, a splicing region, a poly(A) insertion site, etc., followed by introduction into the eucaryotic cells. The expression vector is exemplified by pKA1, pED6dpc2, pCDM8, pSVK3, pMSG, pSVL, pBK-CMV, pBK-RSV, EBV vector, pRS, pYES2, and so on. Examples of eucaryotic cells to be used in general include mammalian culture cells such as simian kidney cells COS7, Chinese hamster ovary cells CHO, etc., budding yeasts, fission yeasts, silkworm cells, *Xenopus laevis* egg cells, and so on, but any eucaryotic cells may be used, provided that they are capable of expressing the present proteins on the membrane surface. The expression vector can be introduced in the eucaryotic cells by methods known in the art such as the electroporation method, the potassium phosphate method, the liposome method, the DEAE-dextran method, and so on.

After one of the proteins of the present invention is expressed in prokaryotic cells or eucaryotic cells, the

objective protein can be isolated from the culture and purified by a combination of separation procedures known in the art. Such examples include treatment with a denaturing agent such as urea or a surface-active agent, sonication, enzymatic digestion, salting-out or solvent precipitation, dialysis, centrifugation, ultrafiltration, gel filtration, SDS-PAGE, isoelectric focusing, ion-exchange chromatography, hydrophobic chromatography, affinity chromatography, reverse phase chromatography, and so on.

The proteins of the present invention include peptide fragments (more than 5 amino acid residues) containing any partial amino acid sequence in the amino acid sequences represented by Sequence Nos. 1 to 9. These peptide fragments can be utilized as antigens for preparation of antibodies. Hereupon, among the proteins of the present invention, those having the signal sequence are secreted in the form of maturation proteins on the surface of the cells, after the signal sequences are removed. Therefore, these maturation proteins shall come within the scope of the present invention. The N-terminal amino acid sequences of the maturation proteins can be easily identified by using the method for the cleavage-site determination in a signal sequence [Japanese Patent Kokai Publication No. 1996-187100]. Furthermore, some membrane proteins undergo the processing on the cell surface to be converted to the secretory forms. Such proteins or peptides in the secretory forms shall come within the scope of the present invention. When sugar chain-binding sites are present in the amino acid sequences, expression in appropriate eucaryotic cells affords proteins wherein sugar chains are added. Accordingly, such proteins or peptides wherein sugar chains are added shall come within the scope of the present invention.

The DNAs of the present invention include all DNAs coding

for the above-mentioned proteins. Said DNAs can be obtained by using a method by chemical synthesis, a method by cDNA cloning, and so on.

The cDNAs of the present invention can be cloned, for example, from cDNA libraries of the human cell origin. These cDNA are synthesized by using as templates poly(A)$^+$ RNAs extracted from human cells. The human cells may be cells delivered from the human body, for example, by the operation or may be the culture cells. The cDNAs can be synthesized by using any method selected from the Okayama-Berg method [Okayama, H. and Berg, P., Mol. Cell. Biol. 2: 161-170 (1982)], the Gubler-Hoffman method [Gubler, U. and Hoffman, J. Gene 25: 263-269 (1983)], and so on, but it is preferred to use the capping method [Kato, S. et al., Gene 150: 243-250 (1994)], as exemplified in Examples, in order to obtain a full-length clone in an effective manner. In addition, commercially available, human cDNA libraries can be utilized. Cloning of the cDNAs of the present invention from the cDNA libraries can be carried out by synthesis of an oligonucleotide on the basis of an optional portion in the cDNA base sequences of the present invention, followed by screening using this oligonucleotide as the probe according to the colony or plaque hybridization by a method known in the art. In addition, the cDNA fragments of the present invention can be prepared by synthesis of an oligonucleotide to be hybridized at both termini of the objective cDNA fragment, followed by the usage of this oligonucleotide as the primer for the RT-PCR method from an mRNA isolated from human cells.

The cDNAs of the present invention are characterized by containing either of the base sequences represented by Sequence Nos. 10 to 18 or the base sequences represented by Sequence Nos. 19, 21, 23, 25, 27, 29, 31, 33 and 35. Table 1 summarizes the

clone number (HP number), the cells affording the cDNA, the total base number of the cDNA, and the number of the amino acid residues of the encoded protein, for each of the cDNAs.

Table 1

Sequence No.	HP No.	Cell	Number of bases	Number of amino acids
1, 10, 19	HP02000	Liver	1705	268
2, 11, 20	HP02061	Saos-2	1759	236
3, 12, 21	HP02163	Saos-2	1069	261
4, 13, 22	HP02219	Stomach Cancer	1759	328
5, 14, 23	HP02256	Stomach Cancer	1697	300
6, 15, 24	HP10390	Stomach Cancer	814	182
7, 16, 25	HP10474	Saos-2	511	66
8, 17, 26	HP10527	Saos-2	1126	183
9, 18, 27	HP10528	Saos-2	2015	324

Hereupon, the same clones as the cDNAs of the present invention can be easily obtained by screening of the cDNA libraries constructed from the human cell lines and human tissues utilized in the present invention by the use of an oligonucleotide probe synthesized on the basis of the cDNA base sequence described in any of Sequence Nos. 10 to 19, 21, 23, 25, 27, 29, 31, 33 and 35.

In general, the polymorphism due to the individual difference is frequently observed in human genes. Accordingly, any cDNA that is subjected to insertion or deletion of one or plural nucleotides and/or substitution with other nucleotides in Sequence Nos. 10 to 19, 21, 23, 25, 27, 29, 31, 33 and 35 shall come within the scope of the present invention.

In a similar manner, any protein that is formed by these modifications comprising insertion or deletion of one or plural amino acids and/or substitution with other amino acids shall

come within the scope of the present invention, as far as the protein possesses the activity of any protein having the amino acid sequences represented by Sequence Nos. 1 to 9.

The cDNAs of the present invention include cDNA fragments (more than 10 bp) containing any partial base sequence in the base sequences represented by Sequence Nos. 10 to 18 or in the base sequences represented by Sequence Nos. 19, 21, 23, 25, 27, 29, 31, 33 and 35. Also, DNA fragments consisting of a sense chain and an anti-sense chain shall come within this scope. These DNA fragments can be utilized as the probes for the gene diagnosis.

In addition to the activities and uses described above, the polynucleotides and proteins of the present invention may exhibit one or more of the uses or biological activities (including those associated with assays cited herein) identified below. Uses or activities described for proteins of the present invention may be provided by administration or use of such proteins or by administration or use of polynucleotides encoding such proteins (such as, for example, in gene therapies or vectors suitable for introduction of DNA).

Research Uses and Utilities

The polynucleotides provided by the present invention can be used by the research community for various purposes. The polynucleotides can be used to express recombinant protein for analysis, characterization or therapeutic use; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in disease states); as molecular weight markers on Southern gels; as chromosome markers or tags (when labeled) to identify chromosomes or to map related gene positions; to compare with endogenous DNA sequences in patients to identify potential genetic disorders;

as probes to hybridize and thus discover novel, related DNA sequences; as a source of information to derive PCR primers for genetic fingerprinting; as a probe to "subtract-out" known sequences in the process of discovering other novel polynucleotides; for selecting and making oligomers for attachment to a "gene chip" or other support, including for examination of expression patterns; to raise anti-protein antibodiesusing DNA immunization techniques; and as an antigen to raise anti-DNA antibodies or elicit another immune response. Where the polynucleotide encodes a protein which binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the polynucleotide can also be used in interaction trap assays (such as, for example, that described in Gyuris et al., Cell 75:791-803 (1993)) to identify polynucleotides encoding the other protein with which binding occurs or to identify inhibitors of the binding interaction.

The proteins provided by the present invention can similarly be used in assay to determine biological activity, including in a panel of multiple proteins for high-throughput screening; to raise antibodies or to elicit another immune response; as a reagent (including the labeled reagent) in assays designed to quantitatively determine levels of the protein (or its receptor) in biological fluids; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in a disease state); and, of course, to isolate correlative receptors or ligands. Where the protein binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the protein can be used to identify the other protein with which binding occurs or to identify inhibitors of the binding interaction. Proteins involved in these binding interactions

can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction.

Any or all of these research utilities are capable of being developed into reagent grade or kit format for commercialization as research products.

Methods for performing the uses listed above are well known to those skilled in the art. References disclosing such methods include without limitation "Molecular Cloning: A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory Press, Sambrook, J., E.F. Fritsch and T. Maniatis eds., 1989, and "Methods in Enzymology: Guide to Molecular Cloning Techniques", Academic Press, Berger, S.L. and A.R. Kimmel eds., 1987.

Nutritional Uses

Polynucleotides and proteins of the present invention can also be used as nutritional sources or supplements. Such uses include without limitation use as a protein or amino acid supplement, use as a carbon source, use as a nitrogen source and use as a source of carbohydrate. In such cases the protein or polynucleotide of the invention can be added to the feed of a particular organism or can be administered as a separate solid or liquid preparation, such as in the form of powder, pills, solutions, suspensions or capsules. In the case of microorganisms, the protein or polynucleotide of the invention can be added to the medium in or on which the microorganism is cultured.

Cytokine and Cell Proliferation/Differentiation Activity

A protein of the present invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one

or more factor dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a protein of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+ (preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e and CMK.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for T-cell or thymocyte proliferation include without limitation those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Bertagnolli et al., J. Immunol. 145:1706-1712, 1990; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Bertagnolli, et al., J. Immunol. 149:3778-3783, 1992; Bowman et al., J. Immunol. 152: 1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: Polyclonal T cell stimulation, Kruisbeek, A.M. and Shevach, E.M. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 3.12.1-3.12.14, John Wiley and Sons, Toronto. 1994; and Measurement of mouse and human Interferon γ, Schreiber, R.D. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto. 1994.

Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without

limitation, those described in: Measurement of Human and Murine Interleukin 2 and Interleukin 4, Bottomly, K., Davis, L.S. and Lipsky, P.E. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto. 1991; deVries et al., J. Exp. Med. 173:1205-1211, 1991; Moreau et al., Nature 336:690-692, 1988; Greenberger et al., Proc. Natl. Acad. Sci. U.S.A. 80:2931-2938, 1983; Measurement of mouse and human interleukin 6-Nordan, R. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto. 1991; Smith et al., Proc. Natl. Acad. Sci. U.S.A. 83:1857-1861, 1986; Measurement of human Interleukin 11 - Bennett, F., Giannotti, J., Clark, S.C. and Turner, K. J. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto. 1991; Measurement of mouse and human Interleukin 9 - Ciarletta, A., Giannotti, J., Clark,S.C. and Turner, K.J. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto. 1991.

Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function; Chapter 6, Cytokines and their cellular receptors; Chapter 7, Immunologic studies in Humans); Weinberger et al., Proc. Natl. Acad. Sci. USA 77:6091-6095, 1980; Weinberger et al., Eur. J. Immun. 11:405-411, 1981; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988.

Immune Stimulating or Suppressing Activity

A protein of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e.g., HIV) as well as bacterial orfungal infections, or may result from autoimmune disorders. More specifically, infectious diseases causes by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpesviruses, mycobacteria, Leishmania spp., malaria spp. and various fungal infections such as candidiasis. Of course, in this regard, a protein of the present invention may also be useful where a boost to the immune system generally may be desirable, i.e., in the treatment of cancer.

Autoimmune disorders which may be treated using a protein of the present invention include, for example, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitis, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein of the present invention may also to be useful in the treatment of allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, organ transplantation), may

also be treatable using a protein of the present invention.

Using the proteins of the invention it may also be possible to immune responses, in a number of ways. Down regulation may be in the form of inhibiting or blocking an immune response already in progress or may involve preventing the induction of an immune response. The functions of activated T cells may be inhibited by suppressing T cell responses or by inducing specific tolerance in T cells, or both. Immunosuppression of T cell responses is generally an active, non-antigen-specific, process which requires continuous exposure of the T cells to the suppressive agent. Tolerance, which involves inducing non-responsiveness or anergy in T cells, is distinguishable from immunosuppression in that it is generally antigen-specific and persists after exposure to the tolerizing agent has ceased. Operationally, tolerance can be demonstrated by the lack of a T cell response upon reexposure to specific antigen in the absence of the tolerizing agent.

Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions (such as , for example, B7)), e.g., preventing high level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of a molecule which inhibits or blocks interaction of a B7 lymphocyte antigen with its natural ligand(s) on immune cells (such as a soluble, monomeric form of a peptide having B7-2 activity alone or in conjunction with a monomeric form of a

peptide having an activity of another B lymphocyte antigen (e.g., B7-1, B7-3) or blocking antibody), prior to transplantation can lead to the binding of the molecule to the natural ligand(s) on the immune cells without transmitting the corresponding costimulatory signal. Blocking B lymphocyte antigen function in this matter prevents cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, the lack of costimulation may also be sufficient to anergize the T cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or tolerance in a subject, it may also be necessary to block the function of a combination of B lymphocyte antigens.

The efficacy of particular blocking reagents in preventing organ transplant rejection or GVHD can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4Ig fusion proteins in vivo as described in Lenschow et al., Science 257:789-792 (1992) and Turka et al., Proc. Natl. Acad. Sci USA, 89:11102-11105 (1992). In addition, murine models of GVHD (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 846-847) can be used to determine the effect of blocking B lymphocyte antigen function in vivo on the development of that disease.

Blocking antigen function may also be therapeutically useful for treating autoimmune diseases. Many autoimmune disorders are the result of inappropriate activation of T cells

that are reactive against self tissue and which promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms. Administration of reagents which block costimulation of T cells by disrupting receptor:ligand interactions of B lymphocyte antigens can be used to inhibit T cell activation and prevent production of autoantibodies or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of autoreactive T cells which could lead to long-term relief from the disease. The efficacy of blocking reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythmatosis in MRL/lpr/lpr mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 840-856).

Upregulation of an antigen function (preferably a B lymphocyte antigen function), as a means of up regulating immune responses, may also be useful in therapy. Upregulation of immune responses may be in the form of enhancing an existing immune response or eliciting an initial immune response. For example, enhancing an immune response through stimulating B lymphocyte antigen function may be useful in cases of viral infection. In addition, systemic viral diseases such as influenza, the commoncold, and encephalitis might be alleviated by the administration of stimulatory forms of B lymphocyte antigens systemically.

Alternatively, anti-viral immune responses may be

enhanced in an infected patient by removing T cells from the patient, costimulating the T cells in vitro with viral antigen-pulsed APCs either expressing a peptide of the present invention or together with a stimulatory form of a soluble peptide of the present invention and reintroducing the in vitro activated T cells into the patient. Another method of enhancing anti-viral immune responses would be to isolate infected cells from a patient, transfect them with a nucleic acid encoding a protein of the present invention as described herein such that the cells express all or a portion of the protein on their surface, and reintroduce the transfected cells into the patient. The infected cells would now be capable of delivering a costimulatory signal to, and thereby activate, T cells in vivo.

In another application, up regulation or enhancement of antigen function (preferably B lymphocyte antigen function) may be useful in the induction of tumor immunity. Tumor cells (e.g., sarcoma, melanoma, lymphoma, leukemia, neuroblastoma, carcinoma) transfected with a nucleic acid encoding at least one peptide of the present invention can be administered to a subject to overcome tumor-specific tolerance in the subject. If desired, the tumor cell can be transfected to express a combination of peptides. For example, tumor cells obtained from a patient can be transfected ex vivo with an expression vector directing the expression of a peptide having B7-2-like activity alone, or in conjunction with a peptide having B7-1-like activity and/or B7-3-like activity. The transfected tumor cells are returned to the patient to result in expression of the peptides on the surface of the transfected cell. Alternatively, gene therapy techniques can be used to target a tumor cell for transfection in vivo.

The presence of the peptide of the present invention

having the activity of a B lymphocyte antigen(s) on the surface of the tumor cell provides the necessary costimulation signal to T cells to induce a T cell mediated immune response against the transfected tumor cells. In addition, tumor cells which lack MHC class I or MHC class II molecules, or which fail to reexpress sufficient amounts of MHC class I or MHC class II molecules, can be transfected with nucleic acid encoding all or a portion of (e.g., a cytoplasmic-domain truncated portion) of an MHC class I α chain protein and β_2 microglobulin protein or an MHC class IIα chain protein and an MHC class IIβ chain protein to thereby express MHC class I or MHC class II proteins on the cell surface. Expression of the appropriate class I or class II MHC in conjunction with a peptide having the activity of a B lymphocyte antigen (e.g., B7-1, B7-2, B7-3) induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II associated protein, such as the invariant chain, can also be cotransfected with a DNA encoding a peptide having the activity of a B lymphocyte antigen to promote presentation of tumor associated antigens and induce tumor specific immunity. Thus, the induction of a T cell mediated immune response in a human subject may be sufficient to overcome tumor-specific tolerance in the subject.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Herrmann et al., Proc. Natl. Acad. Sci. USA

78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Bowmanet al., J. Virology 61:1992-1998; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Brown et al., J. Immunol. 153:3079-3092, 1994.

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J. Immunol. 144:3028-3033, 1990; and Assays for B cell function: In vitro antibody production, Mond, J.J. and Brunswick, M. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto. 1994.

Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., J. Immunol. 149:3778-3783, 1992.

Dendritic cell-dependent assays (which will identify, among others, proteins expressed by dendritic cells that

activate naive T-cells) include, without limitation, those described in: Guery et al., J. Immunol. 134:536-544, 1995; Inaba et al., Journal of Experimental Medicine 173:549-559, 1991; Macatonia et al., Journal of Immunology 154:5071-5079, 1995; Porgador et al., Journal of Experimental Medicine 182:255-260, 1995; Nair et al., Journal of Virology 67:4062-4069, 1993; Huang et al., Science 264:961-965, 1994; Macatonia et al., Journal of Experimental Medicine 169:1255-1264, 1989; Bhardwaj et al., Journal of Clinical Investigation 94:797-807, 1994; and Inaba et al., Journal of Experimental Medicine 172:631-640, 1990.

Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz et al., Cytometry 13:795-808, 1992; Gorczyca et al., Leukemia 7:659-670, 1993; Gorczyca et al., Cancer Research 53:1945-1951, 1993; Itoh et al., Cell 66:233-243, 1991; Zacharchuk, Journal of Immunology 145:4037-4045, 1990; Zamai et al., Cytometry 14:891-897, 1993; Gorczyca et al., International Journal of Oncology 1:639-648, 1992.

Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica et al., Blood 84:111-117, 1994; Fine et al., Cellular Immunology 155:111-122, 1994; Galy et al., Blood 85:2770-2778, 1995; Toki et al., Proc. Nat. Acad Sci. USA 88:7548-7551, 1991.

Hematopoiesis Regulating Activity

A protein of the present invention may be useful in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell deficiencies. Even marginal biological activity in support of colony forming cells or of

factor-dependent cell lines indicates involvement in regulating hematopoiesis, e.g. in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells; in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (i.e., traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentary to platelet transfusions; and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either in-vivo or ex-vivo (i.e., in conjunction with bone marrow transplantation or with peripheral progenitor cell transplantation (homologous or heterologous)) as normal cells or genetically manipulated for gene therapy.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

Assays for embryonic stem cell differentiation (which

will identify, among others, proteins that influence embryonic differentiation hematopoiesis) include, without limitation, those described in: Johansson et al. Cellular Biology 15:141-151, 1995; Keller et al., Molecular and Cellular Biology 13:473-486, 1993; McClanahan et al., Blood 81:2903-2915, 1993.

Assays for stem cell survival and differentiation (which will identify, among others, proteins that regulate lympho-hematopoiesis) include, without limitation, those described in: Methylcellulose colony forming assays, Freshney, M.G. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 265-268, Wiley-Liss, Inc., New York, NY. 1994; Hirayama et al., Proc. Natl. Acad. Sci. USA 89:5907-5911, 1992; Primitive hematopoietic colony forming cells with high proliferative potential, McNiece, I.K. and Briddell, R.A. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 23-39, Wiley-Liss, Inc., New York, NY. 1994; Neben et al., Experimental Hematology 22:353-359, 1994; Cobblestone area forming cell assay, Ploemacher, R.E. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 1-21, Wiley-Liss, Inc., New York, NY. 1994; Long term bone marrow cultures in the presence of stromal cells, Spooncer, E., Dexter, M. and Allen, T. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 163-179, Wiley-Liss, Inc., New York, NY. 1994; Long term culture initiating cell assay, Sutherland, H.J. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 139-162, Wiley-Liss, Inc., New York, NY. 1994.

Tissue Growth Activity

A protein of the present invention also may have utility in compositions used for bone, cartilage, tendon, ligament and/or nerve tissue growth or regeneration, as well as for wound healing and tissue repair and replacement, and in the

treatment of burns, incisions and ulcers.

A protein of the present invention, which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Such a preparation employing a protein of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

A protein of this invention may also be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. A protein of the invention may also be useful in the treatment of osteoporosis or osteoarthritis, such as through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes.

Another category of tissue regeneration activity that may be attributable to the protein of the present invention is tendon/ligament formation. A protein of the present invention, which induces tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed, has application in the healing of tendon or ligament tears, deformities and other tendon or ligament defects in humans and other animals. Such a preparation employing a tendon/ligament-like tissue inducing protein may have prophylactic use in preventing damage to tendon or ligament

tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing defects to tendon or ligament tissue. De novo tendon/ligament-like tissue formation induced by a composition of the present invention contributes to the repair of congenital, trauma induced, or other tendon or ligament defects of other origin, and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments. The compositions of the present invention may provide an environment to attract tendon or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon/ligament cells or progenitors ex vivo for return in vivo to effect tissue repair. The compositions of the invention may also be useful in the treatment of tendinitis, carpal tunnel syndrome and other tendon or ligament defects. The compositions may also include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

The protein of the present invention may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue, i.e. for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders, which involve degeneration, death or trauma to neural cells or nerve tissue. More specifically, a protein may be used in the treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions which may be treated in accordance with the present invention include mechanical and traumatic disorders,

such as spinal cord disorders, head trauma and cerebrovascular diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical therapies may also be treatable using a protein of the invention.

Proteins of the invention may also be useful to promote better or faster closure of non-healing wounds, including without limitation pressure ulcers, ulcers associated with vascular insufficiency, surgical and traumatic wounds, and the like.

It is expected that a protein of the present invention may also exhibit activity for generation or regeneration of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac) and vascular (including vascular endothelium) tissue, or for promoting the growth of cells comprising such tissues. Part of the desired effects may be by inhibition or modulation of fibrotic scarring to allow normal tissue to regenerate. A protein of the invention may also exhibit angiogenic activity.

A protein of the present invention may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage.

A protein of the present invention may also be useful for promoting or inhibiting differentiation of tissues described above from precursor tissues or cells; or for inhibiting the growth of tissues described above.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for tissue generation activity include, without limitation, those described in: International Patent Publication No. WO95/16035 (bone, cartilage, tendon);

International Patent Publication No. WO95/05846 (nerve, neuronal); International Patent Publication No. WO91/07491 (skin, endothelium).

Assays for wound healing activity include, without limitation, those described in: Winter, Epidermal Wound Healing, pps. 71-112 (Maibach, HI and Rovee, DT, eds.), Year Book Medical Publishers, Inc., Chicago, as modified by Eaglstein and Mertz, J. Invest. Dermatol 71:382-84 (1978).

Activin/Inhibin Activity

A protein of the present invention may also exhibit activin- or inhibin-related activities. Inhibins are characterized by their ability to inhibit the release of follicle stimulating hormone (FSH), while activins and are characterized by their ability to stimulate the release of follicle stimulating hormone (FSH). Thus, a protein of the present invention, alone or in heterodimers with a member of the inhibin α family, may be useful as a contraceptive based on the ability of inhibins to decrease fertility in female mammals and decrease spermatogenesis in male mammals. Administration of sufficient amounts of other inhibins can induce infertility in these mammals. Alternatively, the protein of the invention, as a homodimer or as a heterodimer with other protein subunits of the inhibin-β group, may be useful as a fertility inducing therapeutic, based upon the ability of activin molecules in stimulating FSH release from cells of the anterior pituitary. See, for example, United States Patent 4,798,885. A protein of the invention may also be useful for advancement of the onset of fertility in sexually immature mammals, so as to increase the lifetime reproductive performance of domestic animals such as cows, sheep and pigs.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for activin/inhibin activity include, without limitation, those described in: Vale et al., Endocrinology 91:562-572, 1972; Ling et al., Nature 321:779-782, 1986; Vale et al., Nature 321:776-779, 1986; Mason et al., Nature 318:659-663, 1985; Forage et al., Proc. Natl. Acad. Sci. USA 83:3091-3095, 1986.

Chemotactic/Chemokinetic Activity

A protein of the present invention may have chemotactic or chemokinetic activity (e.g., act as a chemokine) for mammalian cells, including, for example, monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells. Chemotactic and chemokinetic proteins can be used to mobilize or attract a desired cell population to a desired site of action. Chemotactic or chemokinetic proteins provide particular advantages in treatment of wounds and other trauma to tissues, as well as in treatment of localized infections. For example, attraction of lymphocytes, monocytes or neutrophils to tumors or sites of infection may result in improved immune responses against the tumor or infecting agent.

A protein or peptide has chemotactic activity for a particular cell population if it can stimulate, directly or indirectly, the directed orientation or movement of such cell population. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population of cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for chemotactic activity (which will identify proteins that induce or prevent chemotaxis)consist of assays

that measure the ability of a protein to induce the migration of cells across a membrane as well as the ability of a protein to induce the adhesion of one cell population to another cell population. Suitable assays for movement and adhesion include, without limitation, those described in: Current Protocols in Immunology, Ed by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W.Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 6.12, Measurement of alpha and beta Chemokines 6.12.1-6.12.28; Taub et al. J. Clin. Invest. 95:1370-1376, 1995; Lind et al. APMIS 103:140-146, 1995; Muller et al Eur. J. Immunol. 25: 1744-1748; Gruber et al. J. of Immunol. 152:5860-5867, 1994; Johnston et al. J. of Immunol. 153: 1762-1768, 1994.

Hemostatic and Thrombolytic Activity

A protein of the invention may also exhibit hemostatic or thrombolytic activity. As a result,such a protein is expected to be useful in treatment of various coagulation disorders (includinghereditary disorders, such as hemophilias) or to enhance coagulation and other hemostatic events in treating wounds resulting from trauma, surgery or other causes. A protein of the invention may also be useful for dissolving or inhibiting formation of thromboses and for treatment and prevention of conditions resulting therefrom (such as, for example, infarction of cardiac and central nervous system vessels (e.g., stroke).

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assay for hemostatic and thrombolytic activity include, without limitation, those described in: Linet et al., J. Clin. Pharmacol. 26:131-140, 1986; Burdick et al., Thrombosis Res. 45:413-419, 1987; Humphrey et al., Fibrinolysis 5:71-79 (1991); Schaub, Prostaglandins 35:467-474, 1988.

Receptor/Ligand Activity

A protein of the present invention may also demonstrate activity as receptors, receptor ligands or inhibitors or agonists of receptor/ligand interactions. Examples of such receptors and ligands include, without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell-cell interactions and their ligands (including without limitation, cellular adhesion molecules (such as selectins, integrins and their ligands) and receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral immune responses). Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. A protein of the present invention (including, without limitation, fragments of receptors and ligands) may themselves be useful as inhibitors of receptor/ligand interactions.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for receptor-ligand activity include without limitation those described in:Current Protocols in Immunology, Ed by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W.Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 7.28, Measurement of Cellular Adhesion under static conditions 7.28.1-7.28.22), Takai et al., Proc. Natl. Acad. Sci. USA 84:6864-6868, 1987; Bierer et al., J. Exp. Med. 168:1145-1156, 1988; Rosenstein et al., J. Exp. Med. 169:149-160 1989; Stoltenborg et al., J. Immunol. Methods 175:59-68, 1994; Stitt et al., Cell 80:661-670, 1995.

Anti-Inflammatory Activity

Proteins of the present invention may also exhibit anti-

inflammatory activity. The anti-inflammatory activity may be achieved by providing a stimulus to cells involved in the inflammatory response, by inhibiting or promoting cell-cell interactions (such as, for example, cell adhesion), by inhibiting or promoting chemotaxis of cells involved in the inflammatory process, inhibiting or promoting cell extravasation, or by stimulating or suppressing production of other factors which more directly inhibit or promote an inflammatory response. Proteins exhibiting such activities can be used to treat inflammatory conditions including chronic or acute conditions), including without limitation inflammation associated with infection (such as septic shock, sepsis or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine-induced lung injury, inflammatory bowel disease, Crohn's disease or resulting from over production of ytokines such as TNF or IL-1. Proteins of the invention may also be useful to treat anaphylaxis and hypersensitivity to an antigenic substance or material.

Tumor Inhibition Activity

In addition to the activities described above for immunological treatment or prevention of tumors, a protein of the invention may exhibit other anti-tumor activities. A protein may inhibit tumor growth directly or indirectly (such as, for example, via ADCC). A protein may exhibit its tumor inhibitory activity by acting on tumor tissue or tumor precursor tissue, by inhibiting formation of tissues necessary to support tumor growth (such as, for example, by inhibiting angiogenesis), by causing production of other factors, agents or cell types which inhibit tumor growth, or by suppressing,

eliminating or inhibiting factors, agents or cell types which promote tumor growth

Other Activities

A protein of the invention may also exhibit one or more of the following additional activities or effects: inhibiting the growth, infection or function of, or killing, infectious agents, including, without limitation, bacteria, viruses, fungi and other parasites; effecting (suppressing or enhancing) bodily characteristics, including, without limitation, height, weight, hair color, eye color, skin, fat to lean ratio or other tissue pigmentation, or organ or body part size or shape (such as, for example, breast augmentation or diminution, change in bone form or shape); effecting biorhythms or caricadic cycles or rhythms; effecting the fertility of male or female subjects; effecting the metabolism, catabolism, anabolism, processing, utilization, storage or elimination of dietary fat, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional factors or component(s); effecting behavioral characteristics, including, without limitation, appetite, libido, stress, cognition (including cognitive disorders), depression (including depressive disorders) and violent behaviors; providing analgesic effects or other pain reducing effects; promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic lineages; hormonal or endocrine activity; in the case of enzymes, correcting deficiencies of the enzyme and treating deficiency-related diseases; treatment of hyperproliferative disorders (such as, for example, psoriasis); immunoglobulin-like activity (such as, for example, the ability to bind antigens or complement); and the ability to act as an antigen in a vaccine composition to raise an immune response against such protein or another

material or entity which is cross-reactive with such protein.

Examples

The present invention is embodied in more detail by the following examples, but this embodiment is not intended to restrict the present invention. The basic operations and the enzyme reactions with regard to the DNA recombination are carried out according to the literature ["Molecular Cloning. A Laboratory Manual", Cold Spring Harbor Laboratory, 1989]. Unless otherwise stated, restrictive enzymes and a variety of modification enzymes to be used were those available from TAKARA SHUZO. The manufacturer's instructions were used for the buffer compositions as well as for the reaction conditions, in each of the enzyme reactions. The cDNA synthesis was carried out according to the literature [Kato, S. et al., Gene 150: 243-250 (1994)].

(1) Preparation of Poly(A)$^+$ RNA

The osteosarcoma cell line Saos-2 (ATCC HTB 85), tissues of stomach cancer delivered by the operation, and the liver were used for human cells to extract mRNAs. The cell line was incubated by a conventional procedure.

After about 1 g of the human cells was homogenized in 20 ml of a 5.5 M guanidinium thiocyanate solution, a total mRNA was prepared according to the literature [Okayama, H. et al., "Method in Enzymology", Vol. 164, Academic Press, 1987]. This was subjected to chromatography on oligo(dT)-cellulose column washed with a 20 mM Tris-hydrochloride buffer solution (pH 7.6), 0.5 M NaCl, and 1 mM EDTA to obtain a poly(A)$^+$ RNA according to the above-described literature.

(2) Construction of cDNA Library

Ten micrograms of the above-mentioned poly(A)$^+$ RNA were

dissolved in a 100 mM Tris-hydrochloride buffer solution (pH 8), one unit of an RNase-free, bacterial alkaline phosphatase was added, and the reaction was run at 37°C for one hour. After the reaction solution was subjected to phenol extraction, followed by ethanol precipitation, the resulting pellet was dissolved in a solution containing 50 mM sodium acetate (pH 6), 1 mM EDTA, 0.1% 2-mercaptoethanol, and 0.01% Triton X-100. Thereto was added one unit of a tobacco-origin acid pyrophosphatase (Epicentre Technologies) and a total 100 μl volume of the resulting mixture was reacted at 37°C for one hour. After the reaction solution was subjected to phenol extraction, followed by ethanol precipitation, the resulting pellet was dissolved in water to obtain a solution of a decapped poly(A)$^+$ RNA.

The decapped poly(A)$^+$ RNA and 3 nmol of a chimeric DNA-RNA oligonucleotide (5'-dG-dG-dG-dG-dA-dA-dT-dT-dC-dG-dA-G-G-A-3') were dissolved in a solution containing 50 mM Tris-hydrochloride buffer solution (pH 7.5), 0.5 mM ATP, 5 mM $MgCl_2$, 10 mM 2-mercaptoethanol, and 25% polyethylene glycol, whereto was added 50 units of T4RNA ligase and a total 30 μl volume of the resulting mixture was reacted at 20°C for 12 hours. After the reaction solution was subjected to phenol extraction, followed by ethanol precipitation, the resulting pellet was dissolved in water to obtain a chimeric-oligo-capped poly(A)$^+$ RNA.

After digestion of vector pKA1 (Japanese Patent Kokai Publication No. 1992-117292) developed by the present inventors with KpnI, about 60 dT tails were added using a terminal transferase. A vector primer to be used below was prepared by digestion of this product with EcoRV to remove a dT tail at one side.

After 6 μg of the previously-prepared chimeric-oligo-capped poly(A)$^+$ RNA was annealed with 1.2 μg of the vector

primer, the resulting product was dissolved in a solution containing 50 mM Tris-hydrochloride buffer solution (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol, and 1.25 mM dNTP (dATP + dCTP + dGTP + dTTP), 200 units of a reverse transcriptase (GIBCO-BRL) were added, and the reaction in a total 20 μl volume was run at 42°C for one hour. After the reaction solution was subjected to phenol extraction, followed by ethanol precipitation, the resulting pellet was dissolved in a solution containing 50 mM Tris-hydrochloride buffer solution (pH 7.5), 100 mM NaCl, 10 mM $MgCl_2$, and 1 mM dithiothreitol. Thereto were added 100 units of EcoRI and a total 20 μl volume of the resulting mixture was reacted at 37°C for one hour. After the reaction solution was subjected to phenol extraction, followed by ethanol precipitation, the resulting pellet was dissolved in a solution containing 20 mM Tris-hydrochloride buffer solution (pH 7.5), 100 mM KCl, 4 mM $MgCl_2$, 10 mM $(NH_4)_2SO_4$, and 50 μg/ml of the bovine serum albumin. Thereto were added 60 units of an *Escherichia coli* DNA ligase and the resulting mixture was reacted at 16°C for 16 hours. To the reaction solution were added 2 μl of 2 mM dNTP, 4 units of *Escherichia coli* DNA polymerase I, and 0.1 unit of *Escherichia coli* RNase H and the resulting mixture was reacted at 12°C for one hour and then at 22°C for one hour.

Next, the cDNA-synthesis reaction solution was used for transformation of *Escherichia coli* DH12S (GIBCO-BRL). The transformation was carried out by the electroporation method. A portion of the transformant was sprayed on the 2xYT agar culture medium containing 100 μg/ml ampicillin and the mixture was incubated at 37°C overnight. A colony formed on the agar medium was picked up at random and inoculated on 2 ml of the 2xYT culture medium containing 100 μg/ml ampicillin. After incubation at 37°C overnight, the culture mixture was

centrifuged to separate the mycelia, from which a plasmid DNA was prepared by the alkaline lysis method. The plasmid DNA was subjected to double digestion with EcoRI and NotI, followed by 0.8% agarose gel electrophoresis, to determine the size of the cDNA insert. Furthermore, using the thus-obtained plasmid as a template, the sequence reaction was carried out by using an M13 universal primer labeled with a fluorescent dye and a Taq polymerase (a kit of Applied Biosystems) and then the product was examined with a fluorescent DNA sequencer (Applied Biosystems) to determine an about 400-bp base sequence at the 5'-terminus of the cDNA. The sequence data were filed as the homo/protein cDNA bank database.

(3) Selection of cDNAs Encoding Proteins Having Transmembrane Domains

A base sequence registered in the homo/protein cDNA bank was converted to three frames of amino acid sequences and the presence or absence of an open reading frame (ORF) beginning from the initiation codon was examined. Then, the selection was made for the presence of a signal sequence that is characteristic to a secretory protein at the N-terminus of the portion encoded by the ORF. These clones were sequenced from the both 5' and 3' directions by the use of the deletion method using exonuclease III to determine the whole base sequence. The hydrophobicity/hydrophilicity profiles were obtained for proteins encoded by the ORF by the Kyte-Doolittle method [Kyte, J. & Doolittle, R. F., J. Mol. Biol. 157: 105-132 (1982)] to examine the presence or absence of a hydrophobic region. In the case in which there is a hydrophobic region of a putative transmembrane domain in the amino acid sequence of an encoded protein, this protein was judged as a membrane protein.

(4) Functional Verification of Secretory Signal Sequence or Transmembrane Domains

It was verified by the method described in the literature [Yokoyama-Kobayashi, M. et al., Gene 163: 193-196 (1995)] that the N-terminal hydrophobic region in the secretory protein clone candidate obtained in the above-mentioned steps functions as a secretory signal sequence. First, the plasmid containing the target cDNA was cleaved at an appropriate restriction enzyme site existing at the downstream of the portion expected for encoding the secretory signal sequence. In the case in which this restriction site was a protruding terminus, the site was blunt-ended by the Klenow treatment or treatment with the T4DNA polymerase. Digestion with HindIII was further carried out and a DNA fragment containing the SV40 promoter and a cDNA encoding the secretory signal sequence at the downstream of the promoter was separated by agarose gel electrophoresis. The resulting fragment was inserted between HindIII in pSSD3 (DDBJ/EMBL/GenBank Registration No. AB007632) and a restriction enzyme site selected so as to match with the urokinase-coding frame, thereby constructing a vector expressing a fusion protein of the secretory signal sequence of the target cDNA and the urokinase protease domain.

After *Escherichia coli* (host: JM109) bearing the fusion-protein expression vector was incubated at 37°C for 2 hours in 2 ml of the 2xYT culture medium containing 100 μg/ml of ampicillin, the helper phage M13KO7 (50 μl) was added and the incubation was continued at 37°C overnight. A supernatant separated by centrifugation underwent precipitation with polyethylene glycol to obtain single-stranded phage particles. These particles were suspended in 100 μl of 1 mM Tris-0.1 mM EDTA, pH 8 (TE). Also, there were used as controls suspensions of single-stranded phage particles prepared in the same manner from pSSD3 and from the vector pKA1-UPA containing a full-length cDNA of urokinase [Yokoyama-Kobayashi, M. et al., Gene

163: 193-196 (1995)].

The culture cells originating from the simian kidney, COS7, were incubated at 37°C in the presence of 5% CO_2 in the Dulbecco's modified Eagle's culture medium (DMEM) containing 10% fetal calf albumin. Into a 6-well plate (Nunc Inc., 3 cm in the well diameter) were inoculated 1×10^5 COS7 cells and incubation was carried out at 37°C for 22 hours in the presence of 5% CO_2. After the culture medium was removed, the cell surface was washed with a phosphate buffer solution and then washed again with DMEM containing 50 mM Tris-hydrochloric acid (pH 7.5) (TDMEM). To the resulting cells was added a suspension of 1 μl of the single-stranded phage suspension, 0.6 ml of the DMEM culture medium, and 3 μl of TRANSFECTAM (IBF Inc.) and the resulting mixture was incubated at 37°C for 3 hours in the presence of 5% CO_2. After the sample solution was removed, the cell surface was washed with TDMEM, 2 ml per well of DMEM containing 10% fetal calf albumin was added, and the incubation was carried out at 37°C for 2 days in the presence of 5% CO_2.

To 10 ml of 50 mM phosphate buffer solution (pH 7.4) containing 2% bovine fibrinogen (Miles Inc.), 0.5% agarose, and 1 mM calcium chloride were added 10 units of human thrombin (Mochida Pharmaceutical Co., Ltd.) and the resulting mixture was solidified in a plate of 9 cm in diameter to prepare a fibrin plate. Ten microliters of the culture supernatant of the tansfected COS7 cells were spotted on the fibrin plate, which was incubated at 37°C for 15 hours. In the case in which a clear circle appears on the fibrin plate, it is judged that the cDNA fragment codes for the amino acid sequence functioning as a secretory signal sequence. On the other hand, in case in which a clear circle is not formed, the cells were washed well, then the fibrin sheet was placed on the cells, and incubation was carried out at 37°C for 15 hours. In case in which a clear

portion is formed on the fibrin sheet, it indicates that the urokinase activity was expressed on the cell surface. In other words, the cDNA fragment is judged to code for the transmembrane domains.

(5) Protein Synthesis by In Vitro Translation

The plasmid vector bearing the cDNA of the present invention was used for in vitro transcription/translation with a T_NT rabbit reticulocyte lysate kit (Promega). In this case, [^{35}S]methionine was added to label the expression product with a radioisotope. Each of the reactions was carried out according to the protocols attached to the kit. Two micrograms of the plasmid was reacted at 30°C for 90 minutes in a total 25 μl volume of the reaction solution containing 12.5 μl of T_NT rabbit reticulocyte lysate, 0.5 μl of a buffer solution (attached to kit), 2 μl of an amino acid mixture (methionine-free), 2 μl of [^{35}S]methionine (Amersham) (0.37 MBq/μl), 0.5 μl of T7RNA polymerase, and 20 U of RNasin. To 3 μl of the resulting reaction solution was added 2 μl of the SDS sampling buffer (125 mM Tris-hydrochloric acid buffer, pH 6.8, 120 mM 2-mercaptoethanol, 2% SDS solution, 0.025% bromophenol blue, and 20% glycerol) and the resulting mixture was heated at 95°C for 3 minutes and then subjected to SDS-polyacrylamide gel electrophoresis. The molecular weight of the translation product was determined by carrying out the autoradiography.

(6) Expression by COS7

Escherichia coli bearing the expression vector of the protein of the present invention was infected with helper phage M13KO7 and single-stranded phage particles were obtained by the above-mentioned procedure. The thus-obtained phage was used for introducing each expression vector in the culture cells originating from the simian kidney, COS7. After incubation at 37°C for 2 days in the presence of 5% CO_2, the incubation was

continued for one hour in the culture medium containing [^{35}S]cystine or [^{35}S]methionine. Collection and dissolution of the cells, followed by subjecting to SDS-PAGE, allowed to observe the presence of a band corresponding to the expression product of each protein, which did not exist in the COS7 cells.

(7) Northern Blot Hybridization

Northern blot hybridization was carried out in order to examine the expression pattern in the human tissues. Filters where poly(A)$^+$ RNAs isolated from each of human tissues are blotted were purchased from Clontech. After excision of a cDNA fragment from the objective clone, followed by agarose-gel electrophoresis to isolate the cDNA fragment, labeling with [^{32}P]dCTP (Amersham) was carried out by using a random primer labeling kit (TAKARA SHUZO). The hybridization was carried out by using a solution attached to the blot paper according to the protocol.

(8) Clone Examples

<HP02000> (Sequence Nos. 1, 10, and 19)

Determination of the whole base sequence of the cDNA insert of clone HP02000 obtained from cDNA libraries of human stomach cancer revealed the structure consisting of a 186-bp 5'-nontranslation region, an 807-bp ORF, and a 712-bp 3'-nontranslation region. The ORF codes for a protein consisting of 268 amino acid residues and there existed two putative transmembrane domains. Figure 1 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 31 kDa that was almost identical with the molecular weight of 30,481 predicted from the ORF. When expressed in COS 7 cells, an expression product of about 32 kDa was observed in the membrane fraction.

The search of the protein data base by using the amino acid sequence of the present protein revealed that the protein was analogous to the rat organic cation transporter (EMBL Accession No. Y09945). Table 2 shows the comparison of the amino acid sequence between the human protein of the present invention (HP) and the rat organic cation transporter (RN). Therein, the marks of -, *, and . represent a gap, an amino acid residue identical with the protein of the present invention, and an amino acid residue analogous to the protein of the present invention, respectively. The both proteins possessed a homology of 67.5% in the N-terminal 169 amino acid residues.

Table 2

```
HS MAFEELLSQVGGLGRFQMLHLVFILPSLMLLIPHILLENFAAAIPGHRCWVHMLDNNTGS
   ***..**.***.*****.*...***   ... ** *****.*.**.******..***.* *
RN MAFQDLLNQVGSLGRFQILQMTFILIFNIIISPHSLLENFTAVIPNHRCWVPILDNDTVS
HS GNETGILSEDALLRISIPLDSNLRPEKCRRFVHPQWQLLHLNGTIHSTSEADTEPCVDGW
   **..* **.*.***.******.**********.***.*******. *..*.*********
RN GNDNGNLSQDDLLRVSIPLDSDLRPEKCRRFVQPQWDLLHLNGTFSSVTEPDTEPCVDGW
HS VYDQSYFPSTIVTKWDLVCDYQSLKSVVQFLLLTGMLVGGIIGGHVSDRWLVESARWLII
   ***** * ***.*.*****. ***.*...**.***.***.*. *...**
RN VYDQSTFLSTIITEWDLVCESQSLDSIAKFLFLTGILVGNILYGPLTDRFGRRLILICAS
HS TNKLDEGLKALRKVARTNGIKNAEETLNIEVVRSTMQEELDAAQTKTTVCDLFRNPSMRK

RN LQMAVTETCAAFAPTFLIYCSLRFLAGISFSTVLTNSALLIIEWTRPKFQALATGLLLCA
HS RICILVFLRKKISRKRHKNDCYTKVTKF

RN GAIGQTVLAGLAFTVRNWHHLHLAMSVPIFFLLVPTRWLSESARWLIMTNKLQKGLKELI
```

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that possessed a homology of 90% or more (for example, Accession No. AA680184) in EST, but anv of the sequences was shorter than the present cDNAs and was not found to contain the initiation codon.

An investigation of the expression pattern in the tissues by northern blot hybridization using the cDNA fragment of the present invention has revealed the expression only in the liver.

The rat organic cation transporter has been found as a membrane protein associated with a drug excretion in the kidney [Grundemann, D. et al., Nature 372: 549-552 (1994)]. Accordingly, the protein of the present invention that is its homologue is considered to possess a similar function and can be utilized for the diagnosis and treatment of diseases that are associated with abnormalities of this enzyme. Furthermore, this is considered to be associated with a drug excretion, so that the cells expressing this protein can be used as a tool for designing this drug. In addition, since this protein is expressed specifically in the liver, a substance prepared so as to possess an affinity with this protein can be applied to the drug delivery system to the liver.

<HP02061> (Sequence Nos. 2, 11, and 21)

Determination of the whole base sequence of the cDNA insert of clone HP02061 obtained from cDNA libraries of human osteosarcoma cell line Saos-2 revealed the structure consisting of a 141-bp 5'-nontranslation region, a 711-bp ORF, and a 907-bp 3'-nontranslation region. The ORF codes for a protein consisting of 236 amino acid residues and there existed two putative transmembrane domains. Figure 2 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation

resulted in formation of a translation product of 26 kDa that was almost identical with the molecular weight of 25,593 predicted from the ORF.

The search of the protein data base by using the amino acid sequence of the present protein revealed that the protein was analogous to the human neuroendocrine-specific protein C (PIR Accession No. I60904). Table 3 shows the comparison of the amino acid sequence between the human protein of the present invention (HP) and the human neuroendocrine-specific protein C (PC). Therein, the marks of -, *, and . represent a gap, an amino acid residue identical with the protein of the present invention, and an amino acid residue analogous to the protein of the present invention, respectively. The C-terminal 187 amino acid residues possessed a homology of 59.9% with the human neuroendocrine-specific protein C.

Table 3

```
HS MAEPSAATQSHSISSSSFGAEPSAPGGGGSPGACPALGTKSCSSSCAVHDLIFWRDVKKT
                                                  **..***.*.*
PC                                  MQATADSTKMDCVWSNWKSQAIDLLYWRDIKQT
HS GFVFGTTLIMLLSLAAFSVISVVSYLILALLSVTISFRIYKSVIQAVQKSEEGHPFKAYL
   *.***. *..*.**..***.***.** ** **.**********.*****..*********
PC GIVFGSFILLLFSLTQFSVVSVVAYLALAALSATISFRIYKSVLQAVQKTDEGHPFKAYL
HS DVDITLSSEAFHNYMNAAMVHINRALKLIIRLFLVEDLVDSLKLAVFMWLMTYVGAVFNG
   ...**** *....* .    ..*..** . *****.*******.**.***.*****.***
PC ELEITLSQEQIQKYTDCLQFYVNSTLKELRRLFLVQDLVDSLKFAVLMWLLTYVGALFNG
HS ITLLILAELLIFSVPIVYEKYKTQIDHYVGIARDQTKSIVEKIQAKLPGIAKKKAE
   .***..* . .*..*.** *...***.*.*..*.....*.*****.** **..**
PC LTLLLMAVVSMFTLPVVYVKHQAQIDQYLGLVRTHINAVVAKIQAKIPG-AKRHAE
```

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the presence of sequences that possessed a homology of 90% or more (for example, Accession No. AA362885) in EST, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

<HP02163> (Sequence Nos. 3, 12, and 23)

Determination of the whole base sequence of the cDNA insert of clone HP02163 obtained from cDNA libraries of human osteosarcoma cell line Saos-2 revealed the structure consisting of a 179-bp 5'-nontranslation region, a 786-bp ORF, and a 104-bp 3'-nontranslation region. The ORF codes for a protein consisting of 261 amino acid residues and there existed one putative transmembrane domain. Figure 3 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 30 kDa that was almost identical with the molecular weight of 29,932 predicted from the ORF. When expressed in COS 7 cells, an expression product of about 28 kDa was observed in the membrane fraction.

The search of the protein data base using the amino acid sequence of the present protein has revealed the presence of sequences that were analogous to a yeast hypothetical protein of 29.4 kDa (SWISS-PROT Accession No. P36039). Table 4 shows the comparison of the amino acid sequence between the human protein of the present invention (HP) and the yeast hypothetical protein of 29.4 kDa (SC). Therein, the marks of -, *, and . represent a gap, an amino acid residue identical with the protein of the present invention, and an amino acid residue analogous to the protein of the present invention, respectively.

The both proteins possessed a homology of 33.2% in the entire region.

Table 4

```
HS                                MAGPELLLDSNIRLWVVLPIVIITFFVGMIRHYVSI
                                    .*..***.... **.*** *.  ..*....*. .
SC MTINQHLQQLLFNRIDKTTSSIQQARAPQMLLDDQLKYWVLLPISIVMVLTGVLKQYIMT
HS LL---QSDKKLTQEQVSDSQVLIRSRVLRENGKYIPKQSFLTRK-YYFNN-PEDGFFKKT
   *.    ...  .. .... * * ....* .**  .....* ..* .  .. .*.  . *.
SC LITGSSANEAQPRVKLTEWQYLQWAQLLIGNGGNLSSDAFAAKKEFLVKDLTEERHLAKA
HS KRK-----VVPPSPMTDPTM---LTDMMKGNVTNVLPMILIGGWINMTFSGFVTTKVPFP
   *..        *.*..**.*   . .* ***... .* ..*  *.*  *.**.  ..***
SC KQQDGSQAGEVPNPFNDPSMSNAMMNMAKGNMASFIPQTIIMWWVNHFFAGFIIMQLPFP
HS LTLRFKPMLQQGIELLTLDASWVSSASWYFLNVFGLRSIYSLI-LGQDNAADQSRMMQEQ
   ** .** *** **   .**..**** ****..*.**...*.** *.... . *.
SC LTAKFKEMLQTGIICQDLDVRWVSSISWYFISVLGLNPVYNLIGLNDQDMGIQAGIGGPQ
HS MTGAAMAMPADTNKAFKTEWEALELTDHQWALDDVEEELMAKDLHFEGMFKKELQTSIF

SC APKALHNHRLTKQCMRWLTI
```

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that possessed a homology of 90% or more (for example, Accession No. Z43161) in EST, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

<HP02219> (Sequence Nos. 4, 13, and 25)

Determination of the whole base sequence of the cDNA insert of clone HP02219 obtained from cDNA libraries of human

stomach cancer revealed the structure consisting of a 58-bp 5'-nontranslation region, a 987-bp ORF, and a 714-bp 3'-nontranslation region. The ORF codes for a protein consisting of 328 amino acid residues and there existed one putative transmembrane domain. Figure 4 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 39 kDa that was almost identical with the molecular weight of 37,299 predicted from the ORF. When expressed in COS 7 cells, an expression product of about 39 kDa was observed in the membrane fraction.

The search of the protein data base using the amino acid sequence of the present protein has revealed that the protein was analogous to *Alabidopsis thaliana* dTDP-glucose 4-6-dehydratase homologue (PIR Accession No. S58282). Table 5 shows the comparison of the amino acid sequence between the human protein of the present invention (HP) and the *Alabidopsis thaliana* dTDP-glucose 4-6-dehydratase homologue (AT). Therein, the marks of * and . represent an amino acid residue identical with the protein of the present invention and an amino acid residue analogous to the protein of the present invention, respectively. The both proteins possessed a homology of 57.2% in 145 amino acid residues at the C-terminal region.

Table 5

```
HS    MVSKALLRLVSAVNRRRMKLLLGIALLAYVASVWGNFVNMSFLLNRSIQENGELKIE

AT RVVVTGGAGFVGSHLVDRLMARGDTVIVVDNFFTGRKENVMHHFSNPNFEMIRHDVVEPI
HS SKIEEMVEPLREKIRDLEKSFTQKYPPVKFLSEKDRKRILITGGAGFVGSHLTDKLMMDG

AT LLEVDQIYHLACPASPVHYKFNPVKTIKTNVVGTLNMLGLAKRVGARFLLTSTSEVYGDP
HS HEVTVVDNFFTGRKRNVEHWIGHENFELINHDVVEPLYIEGVEVRVARIFNTFGPRMHMN
                                                ****.******.**** ..
AT LQHPQVETYWGNVNPIGVRSCYDEGKRTAETLTMDYHRGSNVEVRIARIFNTYGPRMCID
HS DGRVVSNFILQALQGEPLTVYGSGSQTRAFQYVSDLVNGLVALMNSNVSSPVNLGNPEEH
   ********. ***. *******.*.***.**.*****.**. **...  .* *****.*
AT DGRVVSNFVAQALRKEPLTVYGDGKQTRSFQFVSDLVEGLMRLMEGEHVGPFNLGNPGEF
HS TILEFAQLIKNLVGSGSEIQFLSEAQDDPQKRKPDIKKAKLMLGWEPVVPLEEGLNKAIH
   *.**.*..... ......*.* ....***.******.*** .***** *.* .**   ..
AT TMLELAKVVQETIDPNANIEFRPNTEDDPHKRKPDITKAKELLGWEPKVSLRQGLPLMVK
HS YFRKELEYQANNQYIPKPKPARIKKGRTRHS
      **. .
AT DFRQRVFGDQKEGSSAAATTTKTTSA
```

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that possessed a homology of 90% or more (for example, Accession No. U46355) in EST, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

<HP02256> (Sequence Nos. 5, 14, and 27)

Determination of the whole base sequence of the cDNA insert of clone HP02256 obtained from cDNA libraries of human stomach cancer revealed the structure consisting of a 131-bp 5'-nontranslation region, a 903-bp ORF, and a 663-bp 3'-nontranslation region. The ORF codes for a protein consisting of 300 amino acid residues and there existed one transmembrane domain at the N-terminus. Figure 5 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 33 kDa that was almost identical with the molecular weight of 32,943 predicted from the ORF. When expressed in COS cells, an expression product of about 30 kDa was observed in the membrane fraction.

The search of the protein data base using the amino acid sequence of the present protein has revealed that the protein was analogous to the Caenorhabditis elegans hypothetical protein T11F9.11 (PID Accession No. 1403260). Table 6 shows the comparison of the amino acid sequence between the human protein of the present invention (HP) and the Caenorhabditis elegans hypothetical protein T11F9.11 (CE). Therein, the marks of -, *, and . represent a gap, an amino acid residue identical with the protein of the present invention, and an amino acid residue analogous to the protein of the present invention, respectively.

The both proteins possessed a homology of 41.7% in the entire region.

Table 6

```
HS MKFLLDILLLLPLLIVCSLESFVKLFIPK---RRKSVTGEIVLITGAGHGIGRLTAYEFA
      **.. ..   .   . .* * *.*.   .****.*. *****.* ***** * ***
CE MDRALDFVKMVVGTLFFIVLNFFKNFLPNGVLPRKSVEGKKVLITGSGSGIGRLMALEFA
HS KLKSKLVLWDINKHGLEETAAKCKGLGAKVHTFVVDCSNREDIYSSAKKVKAEIGDVSIL
   ** ...*.**.**.* *** ..    *.*. ***** *. .**.. **..*...**..**
CE KLGAEVVIWDVNKDGAEETKNQVVKAGGKASTFVVDLSQYKDIHKVAKETKEAVGDIDIL
HS VNNAGVVYTSDLFATQDPQIEKTFEVNVLAHFWTTKAFLPAMTKNNHGHIVTVASAAGHV
   .****.* ...**. .*  .***..**. * *.*.*.***.* ....**.**.** **..
CE INNAGIVTGKKLFDCPDELMEKTMAVNTNALFYTAKNFLPSMLEKDNGHLVTIASMAGKT
HS SVPFLLAYCSSKFAAVGFHKTLTDELAALQITGVKTTCLCPNFVNTG-F--IKNPSTSLG
   .   *..**.** .*.* *.... *. * .  **.** .** *..** *  ... ...*
CE GCVGLVDYCASKHGAIGCHDSIAMEILAQKKYGVNTTLVCPFFIDTGMFHGVTTKCPALF
HS PTLEPEEVVNRLMHGILTEQKMIFIPSSIAFLTTLERILPERFLAVLKRKISVKFDAVIG
   *.**.. .*. ....***.. .. .*..  .. .*  .**
CE PILEANYAVECIVEAILTNRPLLCMPKASYLILALIGLLPIESQVMMADFFGTNESMNDF
HS YKMKAQ

CE KGRQKND
```

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that possessed a homology of 90% or more (for example, Accession No. H61494) in EST, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the

present invention.

<HP10390> (Sequence Nos. 6, 15, and 29)

Determination of the whole base sequence of the cDNA insert of clone HP10390 obtained from cDNA libraries of human stomach cancer revealed the structure consisting of a 144-bp 5'-nontranslation region, a 549-bp ORF, and a 121-bp 3'-nontranslation region. The ORF codes for a protein consisting of 182 amino acid residues and possessed one transmembrane domain in the N-terminus. Figure 6 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. Introduction of an expression vector, wherein the HindIII-BstXI (treated with T4RNA polymerase) fragment containing a cDNA portion coding for the N-terminal 50 amino acid residues of the present protein was inserted into the HindIII-SmaI site of pSSD3, into the COS7 cells revealed the urokinase activity on the surface of the cells to indicate that the present protein is the type-II membrane protein. In vitro translation resulted in formation of a translation product of 20 kDa that was almost identical with the molecular weight of 20,639 predicted from the ORF. When expressed in COS cells, an expression product of about 19 kDa was observed in the supernatant fraction and the membrane fraction.

The search of the protein data base using the amino acid sequence of the present protein has not identified any known protein having an analogy. Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the presence of sequences that possessed a homology of 90% or more (for example, Accession No. AA315322) in EST, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

<HP10474> (Sequence Nos. 7, 16, and 31)

Determination of the whole base sequence of the cDNA insert of clone HP10474 obtained from cDNA libraries of human osteosarcoma cell line Saos-2 revealed the structure consisting of a 22-bp 5'-nontranslation region, a 201-bp ORF, and a 288-bp 3'-nontranslation region. The ORF codes for a protein consisting of 66 amino acid residues and possessed one transmembrane domain at the C-terminus. Figure 7 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 10 kDa that was almost identical with the molecular weight of 7,599 predicted from the ORF.

The search of the protein data base using the amino acid sequence of the present protein has not revealed the presence of any known protein having an analogy. Also, the search of the GenBank using the base sequences of the present cDNA has revealed the presence of sequences that possessed a homology of 90% or more (for example, Accession No. H30340) in EST, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

<HP10527> (Sequence Nos. 8, 17, and 33)

Determination of the whole base sequence of the cDNA insert of clone HP10527 obtained from cDNA libraries of the human osteosarcoma cell line Saos-2 revealed the structure consisting of a 113-bp 5'-nontranslation region, a 552-bp ORF, and a 461-bp 3'-nontranslation region. The ORF codes for a protein consisting of 183 amino acid residues and possessed three putative transmembrane domains. Figure 8 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. As the result of in

vitro translation, there was produced a translation product of about 21 kDa, which is nearly equal to a molecular weight of 21,111 as expected from ORF.

The search of the protein data base using the amino acid sequence of the present protein has not revealed the presence of any known protein having an analogy. Also, the search of the GenBank using the base sequences of the present cDNA has revealed the presence of sequences that possessed a homology of 90% or more (for example, Accession No. AA310892) in EST, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

<HP10528> (Sequence Nos. 9, 18, and 35)

Determination of the whole base sequence of the cDNA insert of clone HP10528 obtained from cDNA libraries of the human osteosarcoma cell line Saos-2 revealed the structure consisting of a 53-bp 5'-nontranslation region, a 975-bp ORF, and a 987-bp 3'-nontranslation region. The ORF codes for a protein consisting of 324 amino acid residues and possessed seven putative transmembrane domains. Figure 9 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. As the result of in vitro translation, there was produced a translation product of about 32 kDa, which is nearly equal to a molecular weight of 34,227 as expected from ORF.

The search of the protein data base using the amino acid sequence of the present protein has revealed it had an analogy to the epithelial cell growth arrest-inducible gene product (PID Accession No. 998569). Table 7 shows the comparison of the amino acid sequence between the human protein of the present invention (HP) and the epithelial cell growth arrest-inducible gene product (GA). Therein, the marks of -, *, and . represent

a gap, an amino acid residue identical with the protein of the present invention, and an amino acid residue analogous to the protein of the present invention, respectively. The both proteins possessed a homology of 34.7% in the entire region.

Table 7

```
HS MGPWGEPELLVWRPEAVASEPPVPVGLEVKLGALVLLLVLTLLCSLVPICVLRRPGANHE
                              *..*** *  **.*** *.*.***  . . ..
GA                          MEQLLGIKLGCLFALLALTLGCGLTPICFKWFQIDAAR
HS GSASRQKALSLVSCFAGGVFLATCLLDLLPDYLAAIDEALAALHV---------------
   *  .*  .*.*..*...****.. .... .. *..*..... . *
GA GHHRR--VLRLLGCISAGVFLGAGFMHMTAEALEEIESQIQKFMVQNRSASERNSSGDAD
HS --TLQFPLQEFILAMGFFLVLVMEQITLAYKEQSGPSPLEETRALLGTVNGGPQHWHDGP
       ...*  *.*...*****. .* ..*..     *.. ..... ..  **.. .
GA SAHMEYPYGELIISLGFFLVFFLESLALQC----CPGA-AGGSTVQDEEWGGAHIF---E
HS GVPQASGAPATPSALRACVLVFSLALHSVFEGLAVGLQRDRARAMELCLALLLHKGILAV
     ...  .... ..*** **..**..************.. * ...****.* ***...
GA LHSHGHLPSPSKGPLRALVLLLSLSFHSVFEGLAVGLQPTVAATVQLCLAVLAHKGLVVF
HS SLSLRLLQSHLRAQVVAGCGILFSCMTPLGIGLGAALAES-AGPLHQLAQSVLEGMAAGT
   ....**..   ... .. . .*.. *.***...* *.... ..  . ***.****.****
GA GVGMRLVHLGTSSRWAVFSILLLALMSPLGLAVGLAVTGGDSEGGRGLAQAVLEGVAAGT
HS FLYITFLEILPQELASSEQRILKVILLLAGFALLTGLLFIQI
    **.*******.****.*... *
GA ELYVTFLEILPRELASPEAPLAKWSCVAAGEAFMAFIALWA
```

The search of the protein data base using the amino acid sequence of the present protein has not revealed the presence of any known protein having an analogy. Also, the search of the GenBank using the base sequences of the present cDNA has revealed the presence of sequences that possessed a homology of

90% or more (for example, Accession No. AA206511) in EST, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

INDUSTRIAL APPLICABILITY

The present invention provides human proteins having transmembrane domains, cDNAs coding for these proteins, and expression vectors of said cDNAs as well as eucaryotic cells expressing said cDNAs. All of the proteins of the present invention exist in the cell membrane, so that they are considered to be proteins controlling the proliferation and the differentiation of the cells. Accordingly, the proteins of the present invention can be employed as pharmaceuticals such as carcinostatic agents relating to the control of the proliferation and the differentiation of the cells or as antigens for preparing antibodies against said proteins. The cDNAs of the present invention can be utilized as probes for the gene diagnosis and gene sources for the gene therapy. Furthermore, the cDNAs can be utilized for large-scale expression of said proteins. Cells, wherein these membrane protein genes are introduced to possess said proteins on the membrane surface, can be utilized for detection of the corresponding ligands, screening of novel low-molecular pharmaceuticals, and so on.

The present invention also provides genes corresponding to the polynucleotide sequences disclosed herein. "Corresponding genes" are the regions of the genome that are transcribed to produce the mRNAs from which cDNA polynucleotide sequences are derived and may include contiguous regions of the genome necessary for the regulated expression of such genes. Corresponding genes may therefore include but are not limited

to coding sequences, 5' and 3' untranslated regions, alternatively spliced exons, introns, promoters, enhancers, and silencer or suppressor elements. The corresponding genes can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include the preparation of probes or primers from the disclosed sequence information for identification and/or amplification of genes in appropriate genomic libraries or other sources of genomic materials. An "isolated gene" is a gene that has been separated from the adjacent coding sequences, if any, present in the genome of the organism from which the gene was isolated.

Organisms that have enhanced, reduced, or modified expression of the gene(s) corresponding to the polynucleotide sequences disclosed herein are provided. The desired change in gene expression can be achieved through the use of antisense polynucleotides or ribozymes that bind and/or cleave the mRNA transcribed from the gene (Albert and Morris, 1994, Trends Pharmacol. Sci. 15(7): 250-254; Lavarosky et al., 1997, Biochem. Mol. Med. 62(1): 11-22; and Hampel, 1998, Prog. Nucleic Acid Res. Mol. Biol. 58: 1-39; all of which are incorporated by reference herein). Transgenic animals that have multiple copies of the gene(s) corresponding to the polynucleotide sequences disclosed herein, preferably produced by transformation of cells with genetic constructs that are stably maintained within the transformed cells and their progeny, are provided. Transgenic animals that have modified genetic control regions that increase or reduce gene expression levels, or that change temporal or spatial patterns of gene expression, are also provided (see European Patent No. 0 649 464 B1, incorporated by reference herein). In addition, organisms are provided in which the gene(s) corresponding to the polynucleotide sequences disclosed herein have been partially

or completely inactivated, through insertion of extraneous sequences into the corresponding gene(s) or through deletion of all or part of the corresponding gene(s). Partial or complete gene inactivation can be accomplished through insertion, preferably followed by imprecise excision, of transposable elements (Plasterk, 1992, Bioessays 14(9): 629-633; Zwaal et al., 1993, Proc. Natl. Acad. Sci. USA 90(16): 7431-7435; Clark et al., 1994, Proc. Natl. Acad. Sci. USA 91(2): 719-722; all of which are incorporated by reference herein), or through homologous recombination, preferably detected by positive/negative genetic selection strategies (Mansour et al., 1988, Nature 336: 348-352; U.S. Patent Nos. 5,464,764; 5,487,992; 5,627,059; 5,631,153; 5,614, 396; 5,616,491; and 5,679,523; all of which are incorporated by reference herein). These organisms with altered gene expression are preferably eukaryotes and more preferably are mammals. Such organisms are useful for the development of non-human models for the study of disorders involving the corresponding gene(s), and for the development of assay systems for the identification of molecules that interact with the protein product(s) of the corresponding gene(s).

Where the protein of the present invention is membrane-bound (e.g., is a receptor), the present invention also provides for soluble forms of such protein. In such forms part or all of the intracellular and transmembrane domains of the protein are deleted such that the protein is fully secreted from the cell in which it is expressed. The intracellular and transmembrane domains of proteins of the invention can be identified in accordance with known techniques for determination of such domains from sequence information.

Proteins and protein fragments of the present invention include proteins with amino acid sequence lengths that are at

least 25%(more preferably at least 50%, and most preferably at least 75%) of the length of a disclosed protein and have at least 60% sequence identity (more preferably, at least 75% identity; most preferably at least 90% or 95% identity) with that disclosed protein, where sequence identity is determined by comparing the amino acid sequences of the proteins when aligned so as to maximize overlap and identity while minimizing sequence gaps. Also included in the present invention are proteins and protein fragments that contain a segment preferably comprising 8 or more (more preferably 20 or more, most preferably 30 or more) contiguous amino acids that shares at least 75% sequence identity (more preferably, at least 85% identity; most preferably at least 95% identity) with any such segment of any of the disclosed proteins.

Species homologs of the disclosed polynucleotides and proteins are also provided by the present invention. As used herein, a "species homologue" is a protein or polynucleotide with a different species of origin from that of a given protein or polynucleotide, but with significant sequence similarity to the given protein or polynucleotide, as determined by those of skill in the art. Species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source from the desired species.

The invention also encompasses allelic variants of the disclosed polynucleotides or proteins; that is, naturally-occurring alternative forms of the isolated polynucleotide which also encode proteins which are identical, homologous, or related to that encoded by the polynucleotides.

The invention also includes polynucleotides with sequences complementary to those of the polynucleotides

disclosed herein.

The present invention also includes polynucleotides capable of hybridizing under reduced stringency conditions, more preferably stringent conditions, and most preferably highlystringent conditions, to polynucleotides described herein. Examples of stringency conditions are shown in the table below: highly stringent conditions are those that are at least as stringent as, for example, conditions A-F; stringent conditions are at least as stringent as, for example, conditions G-L; and reduced stringency conditions are at least as stringent as, for example, conditions M-R.

Table

Stringency Condition	Polynucleotide Hybrid	Hybrid Length (bp)‡	Hybridization Temperature and Buffer†	Wash Temperature and Buffer†
A	DNA : DNA	≥50	65℃; 1×SSC -or- 42℃; 1×SSC,50% formamide	65℃; 0.3×SSC
B	DNA : DNA	<50	T_B*; 1×SSC	T_B*; 1×SSC
C	DNA : RNA	≥50	67℃; 1×SSC -or- 45℃; 1×SSC,50% formamide	67℃; 0.3×SSC
D	DNA : RNA	<50	T_D*; 1×SSC	T_D*; 1×SSC
E	RNA : RNA	≥50	70℃; 1×SSC -or- 50℃; 1×SSC,50% formamide	70℃; 0.3×SSC
F	RNA : RNA	<50	T_F*; 1×SSC	T_F*; 1×SSC
G	DNA : DNA	≥50	65℃; 4×SSC -or- 42℃; 4×SSC,50% formamide	65℃; 1×SSC
H	DNA : DNA	<50	T_H*; 4×SSC	T_H*; 4×SSC
I	DNA : RNA	≥50	67℃; 4×SSC -or- 45℃; 4×SSC,50% formamide	67℃; 1×SSC
J	DNA : RNA	<50	T_J*; 4×SSC	T_J*; 4×SSC
K	RNA : RNA	≥50	70℃; 4×SSC -or- 50℃; 4×SSC,50% formamide	67℃; 1×SSC
L	RNA : RNA	<50	T_L*; 2×SSC	T_L*; 2×SSC
M	DNA : DNA	≥50	50℃; 4×SSC -or- 40℃; 6×SSC,50% formamide	50℃; 2×SSC
N	DNA : DNA	<50	T_N*; 6×SSC	T_N*; 6×SSC
O	DNA : RNA	≥50	55℃; 4×SSC -or- 42℃; 6×SSC,50% formamide	55℃; 2×SSC
P	DNA : RNA	<50	T_P*; 6×SSC	T_P*; 6×SSC
Q	RNA : RNA	≥50	60℃; 4×SSC -or- 45℃; 6×SSC,50% formamide	60℃; 2×SSC
R	RNA : RNA	<50	T_R*; 4×SSC	T_R*; 4×SSC

‡ : The hybrid length is that anticipated for the hybridized region(s) of the hybridizing polynucleotides. When hybridizing a polynucleotide to a target polynucleotide of unknown sequence, the hybrid length is assumed to be that of the hybridizing polynucleotide. When polynucleotides of known sequence are hybridized, the hybrid

length can be determined by aligning the sequences of the polynucleotides and identifying the region or regions of optimal sequence complementarity.
†: SSPE (1×SSPE is 0.15M NaCl, 10mM NaH_2PO_4, and 1.25mM EDTA, pH7.4) can be substituted for SSC (1×SSC is 0.15M NaCl and 15mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes after hybridization is complete.
*T_B - T_R: The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature (T_m) of the hybrid, where T_m is determined according to the following equations. For hybrids less than 18 base pairs in length, T_m(°C)=2(#of A + T bases) + 4(# of G + C bases). For hybrids between 18 and 49 base pairs in length, T_m(°C)=81.5 + 16.6($\log_{10}$[Na^+]) + 0.41 (%G+C) - (600/N), where N is the number of bases in the hybrid, and [Na^+] is the concentration of sodium ions in the hybridization buffer ([Na^+] for 1×SSC=0.165M).

Additional examples of stringency conditions for polynucleotide hybridization are provided in Sambrook, J., E.F. Fritsch, and T. Maniatis, 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, chapters 9 and 11, and Current Protocols in Molecular Biology, 1995, F.M. Ausubel et al., eds., John Wiley & Sons, Inc., sections 2.10 and 6.3-6.4, incorporated herein by reference.

Preferably, each such hybridizing polynucleotide has a length that is at least 25%(more preferably at least 50%, and most preferably at least 75%) of the length of the polynucleotide of the present invention to which it hybridizes, and has at least 60% sequence identity (more preferably, at least 75% identity; most preferably at least 90% or 95% identity) with the polynucleotide of the present invention to which it hybridizes, where sequence identity is determined by comparing the sequences of the hybridizing polynucleotides when aligned so as to maximize overlap and identity while minimizing sequence gaps.

CLAIMS

1. A protein comprising any of the amino acid sequences represented by Sequence Nos. 1 to 9.

2. A DNA coding for any of the proteins as claimed in Claim 1.

3. A cDNA comprising any of the base sequences represented by Sequence Nos. 10 to 18.

4. The cDNA as claimed in Claim 3 comprising any of the base sequences represented by Sequence Nos. 19, 21, 23, 25, 27, 29, 31, 33 and 35.

5. An expression vector capable of expressing the DNA as claimed in any of Claim 2 to Claim 4 by in vitro translation or in eucaryotic cells.

6. A transformation eucaryotic cell capable of expressing the DNA as claimed in any of Claim 2 to Claim 4 and producing the protein as claimed in Claim 1.

526 Rec'd PCT/PTO 27 OCT 2000
PCT/JP99/02226

09/674235

Sequence listing

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         35                  40                  45
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     50                  55                  60
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Trp Leu Ile Ile Thr Asn Lys Leu Asp Glu Gly Leu Lys Ala Leu Arg
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Lys Val Ala Arg Thr Asn Gly Ile Lys Asn Ala Glu Glu Thr Leu Asn
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Gly Thr Thr Leu Ile Met Leu Leu Ser Leu Ala Ala Phe Ser Val Ile
 65                  70                  75                  80
Ser Val Val Ser Tyr Leu Ile Leu Ala Leu Leu Ser Val Thr Ile Ser
                85                  90                  95
Phe Arg Ile Tyr Lys Ser Val Ile Gln Ala Val Gln Lys Ser Glu Glu
            100                 105                 110
Gly His Pro Phe Lys Ala Tyr Leu Asp Val Asp Ile Thr Leu Ser Ser
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Ala Leu Val Leu Leu Leu Val Leu Thr Leu Leu Cys Ser Leu Val Pro
35 40 45
Ile Cys Val Leu Arg Arg Pro Gly Ala Asn His Glu Gly Ser Ala Ser
50 55 60
Arg Gln Lys Ala Leu Ser Leu Val Ser Cys Phe Ala Gly Gly Val Phe
65 70 75 80
Leu Ala Thr Cys Leu Leu Asp Leu Leu Pro Asp Tyr Leu Ala Ala Ile
85 90 95
Asp Glu Ala Leu Ala Ala Leu His Val Thr Leu Gln Phe Pro Leu Gln
100 105 110
Glu Phe Ile Leu Ala Met Gly Phe Phe Leu Val Leu Val Met Glu Gln
115 120 125
Ile Thr Leu Ala Tyr Lys Glu Gln Ser Gly Pro Ser Pro Leu Glu Glu
130 135 140
Thr Arg Ala Leu Leu Gly Thr Val Asn Gly Gly Pro Gln His Trp His
145 150 155 160
Asp Gly Pro Gly Val Pro Gln Ala Ser Gly Ala Pro Ala Thr Pro Ser
165 170 175
Ala Leu Arg Ala Cys Val Leu Val Phe Ser Leu Ala Leu His Ser Val
180 185 190
Phe Glu Gly Leu Ala Val Gly Leu Gln Arg Asp Arg Ala Arg Ala Met
195 200 205
Glu Leu Cys Leu Ala Leu Leu Leu His Lys Gly Ile Leu Ala Val Ser
210 215 220
Leu Ser Leu Arg Leu Leu Gln Ser His Leu Arg Ala Gln Val Val Ala
225 230 235 240
Gly Cys Gly Ile Leu Phe Ser Cys Met Thr Pro Leu Gly Ile Gly Leu
245 250 255

Gly Ala Ala Leu Ala Glu Ser Ala Gly Pro Leu His Gln Leu Ala Gln
260 265 270
Ser Val Leu Glu Gly Met Ala Ala Gly Thr Phe Leu Tyr Ile Thr Phe
275 280 285
Leu Glu Ile Leu Pro Gln Glu Leu Ala Ser Ser Glu Gln Arg Ile Leu
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Lys Val Ile Leu Leu Leu Ala Gly Phe Ala Leu Leu Thr Gly Leu Leu
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Phe Ile Gln Ile

<210> 10
<211> 804
<212> DNA
<213> Homo sapiens

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gctgcagcca ttcctggtca tcgttgctgg gtccacatgc tggacaataa tactggatct 180
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tcaaatctga ggccagagaa gtgtcgtcgc tttgtccatc cccagtggca gcttcttcac 300
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gtatatgatc aaagctactt cccttcgacc attgtgacta agtgggacct ggtatgtgat 420
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accaataaac tagatgaggg cttaaaggca cttagaaaag ttgcacgcac aaatggaata 600
aagaatgctg aagaaaccct gaacatagag gttgtaagat ccaccatgca ggaggagctg 660
gatgcagcac agaccaaaac tactgtgtgt gacttgttcc gcaaccccag tatgcgtaaa 720
aggatctgta tcctggtatt tttgagaaaa aaaatctcaa ggaaaaggca taaaaatgat 780
tgctacacaa aagtgaccaa attt 804

<210> 11
<211> 708
<212> DNA
<213> Homo sapiens

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agctgcagct cctcctgtgc ggtgcacgat ctgattttct ggagagatgt gaagaagact 180
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<210> 12
<211> 783
<212> DNA
<213> Homo sapiens

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gacaagaagc tcacccagga acaagtatct gacagtcaag tcctaattcg aagcagagtc 180
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gagcagatga cgggagcagc catggccatg cccgcagaca caaacaaagc tttcaagaca 660
gagtgggaag ctttggagct gacggatcac cagtgggcac tagatgatgt cgaagaagag 720
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ttt 783

<210> 13
<211> 984
<212> DNA
<213> Homo sapiens

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agcttttctac tcaacaggtc tatccaggaa aatggtgaac taaaaattga aagcaagatt 180
gaagagatgg ttgaaccact aagagagaaa atcagagatt tagaaaaaag ctttacccag 240
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ggcgcagggt tcgtgggctc ccatctaact gacaaactca tgatggacgg ccacgaggtg 360
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gtgcgagtgg ccagaatctt caacaccttt gggccacgca tgcacatgaa cgatgggcga 540
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aaagaactcg agtaccaggc aaataatcag tacatcccca aaccaaagcc tgccagaata 960
aagaaaggac ggactcgcca cagc 984

<210> 14
<211> 900
<212> DNA
<213> Homo sapiens

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attacaggag ctgggcatgg aattgggaga ctgactgcct atgaatttgc taaacttaaa 180
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gatgaactgg ctgccttaca aataactgga gtcaaaacaa catgtctgtg tcctaatttc 660
gtaaacactg gcttcatcaa aaatccaagt acaagtttgg gacccactct ggaacctgag 720
gaagtggtaa acaggctgat gcatgggatt ctgactgagc agaagatgat ttttattcca 780
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<210> 15
<211> 546
<212> DNA
<213> Homo sapiens

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gatggcagcc agtcagtggt ggaggtgcct tatgcccgct cagaggccca cctcacagag 240
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gtggaggaat acgaggatga actcattgaa ttcttttccc gagaggctga caatgttaaa 480
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gagcta

<210> 16
<211> 198
<212> DNA
<213> Homo sapiens

<400> 16

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aaacactcct actggttgga cctctggctt ttcatccttt tcgatgtggt ggtgtttctc 180
tttgtgtatt ttttgcca 198

<210> 17
<211> 549
<212> DNA
<213> Homo sapiens

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gtgctctcca tgatcagcat gggactcttt tccatcgctc cactcattta tggcagcatg 360
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ggtttttctg ccgtttccat catgtacctg gtgttggtgt tggcagtgca agtgcatgcc 480
tggcagttgt actacagcaa gaagctccta gactcttggt tcaccagcac acaggagaag 540
aagcataaa 549

<210> 18
<211> 972
<212> DNA
<213> Homo sapiens

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accctcctct gcagcctggt gcccatctgt gtgctgcgcc ggccaggagc taaccatgaa 180
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gcagccttgc acgtgacgct ccagttccca ctgcaagagt tcatcctggc catgggcttc 360
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cctctggagg aaacaagggc tctgctggga acagtgaatg gtgggccgca gcattggcat 480
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<210> 19
<211> 1705
<212> DNA
<213> Homo sapiens

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tctccctttg aacttatctc cgtaaagcca ttgtgcctcc tcttgggggt cacgtgttca 180
caatca atg gcc ttt gag gag ctc ttg agt caa gtt gga ggc ctt ggg 228
Met Ala Phe Glu Glu Leu Leu Ser Gln Val Gly Gly Leu Gly
1 5 10
aga ttt cag atg ctt cat ctg gtt ttt att ctt ccc tct ctc atg tta 276
Arg Phe Gln Met Leu His Leu Val Phe Ile Leu Pro Ser Leu Met Leu
15 20 25 30
tta atc cct cat ata ctg cta gag aac ttt gct gca gcc att cct ggt 324
Leu Ile Pro His Ile Leu Leu Glu Asn Phe Ala Ala Ala Ile Pro Gly
35 40 45
cat cgt tgc tgg gtc cac atg ctg gac aat aat act gga tct ggt aat 372
His Arg Cys Trp Val His Met Leu Asp Asn Asn Thr Gly Ser Gly Asn
50 55 60
gaa act gga atc ctc agt gaa gat gcc ctc ttg aga atc tct atc cca 420
Glu Thr Gly Ile Leu Ser Glu Asp Ala Leu Leu Arg Ile Ser Ile Pro
65 70 75

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cta gac tca aat ctg agg cca gag aag tgt cgt cgc ttt gtc cat ccc      468
Leu Asp Ser Asn Leu Arg Pro Glu Lys Cys Arg Arg Phe Val His Pro
     80                  85                  90
cag tgg cag ctt ctt cac ctg aat ggg act atc cac agc aca agt gag      516
Gln Trp Gln Leu Leu His Leu Asn Gly Thr Ile His Ser Thr Ser Glu
 95                 100                 105                 110
gca gac aca gaa ccc tgt gtg gat ggc tgg gta tat gat caa agc tac      564
Ala Asp Thr Glu Pro Cys Val Asp Gly Trp Val Tyr Asp Gln Ser Tyr
                115                 120                 125
ttc cct tcg acc att gtg act aag tgg gac ctg gta tgt gat tat cag      612
Phe Pro Ser Thr Ile Val Thr Lys Trp Asp Leu Val Cys Asp Tyr Gln
            130                 135                 140
tca ctg aaa tca gtg gtt caa ttc cta ctt ctg act gga atg ctg gtg      660
Ser Leu Lys Ser Val Val Gln Phe Leu Leu Leu Thr Gly Met Leu Val
        145                 150                 155
gga ggc atc ata ggt ggc cat gtc tca gac agg tgg ctg gtg gaa tct      708
Gly Gly Ile Ile Gly Gly His Val Ser Asp Arg Trp Leu Val Glu Ser
    160                 165                 170
gct cgg tgg ttg ata atc acc aat aaa cta gat gag ggc tta aag gca      756
Ala Arg Trp Leu Ile Ile Thr Asn Lys Leu Asp Glu Gly Leu Lys Ala
175                 180                 185                 190
ctt aga aaa gtt gca cgc aca aat gga ata aag aat gct gaa gaa acc      804
Leu Arg Lys Val Ala Arg Thr Asn Gly Ile Lys Asn Ala Glu Glu Thr
                195                 200                 205
ctg aac ata gag gtt gta aga tcc acc atg cag gag gag ctg gat gca      852
Leu Asn Ile Glu Val Val Arg Ser Thr Met Gln Glu Glu Leu Asp Ala
            210                 215                 220
gca cag acc aaa act act gtg tgt gac ttg ttc cgc aac ccc agt atg      900
Ala Gln Thr Lys Thr Thr Val Cys Asp Leu Phe Arg Asn Pro Ser Met
        225                 230                 235
cgt aaa agg atc tgt atc ctg gta ttt ttg aga aaa aaa atc tca agg      948
Arg Lys Arg Ile Cys Ile Leu Val Phe Leu Arg Lys Lys Ile Ser Arg
    240                 245                 250
aaa agg cat aaa aat gat tgc tac aca aaa gtg acc aaa ttt taagaagcct  1000
Lys Arg His Lys Asn Asp Cys Tyr Thr Lys Val Thr Lys Phe
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255 260 265

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<210> 20
<211> 268
<212> PRT
<213> Homo sapiens

<400> 20

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Arg Phe Gln Met Leu His Leu Val Phe Ile Leu Pro Ser Leu Met Leu
15 20 25 30
Leu Ile Pro His Ile Leu Leu Glu Asn Phe Ala Ala Ala Ile Pro Gly
35 40 45
His Arg Cys Trp Val His Met Leu Asp Asn Asn Thr Gly Ser Gly Asn
50 55 60
Glu Thr Gly Ile Leu Ser Glu Asp Ala Leu Leu Arg Ile Ser Ile Pro
65 70 75
Leu Asp Ser Asn Leu Arg Pro Glu Lys Cys Arg Arg Phe Val His Pro
80 85 90
Gln Trp Gln Leu Leu His Leu Asn Gly Thr Ile His Ser Thr Ser Glu
95 100 105 110
Ala Asp Thr Glu Pro Cys Val Asp Gly Trp Val Tyr Asp Gln Ser Tyr

115 120 125
Phe Pro Ser Thr Ile Val Thr Lys Trp Asp Leu Val Cys Asp Tyr Gln
130 135 140
Ser Leu Lys Ser Val Val Gln Phe Leu Leu Leu Thr Gly Met Leu Val
145 150 155
Gly Gly Ile Ile Gly Gly His Val Ser Asp Arg Trp Leu Val Glu Ser
160 165 170
Ala Arg Trp Leu Ile Ile Thr Asn Lys Leu Asp Glu Gly Leu Lys Ala
175 180 185 190
Leu Arg Lys Val Ala Arg Thr Asn Gly Ile Lys Asn Ala Glu Glu Thr
195 200 205
Leu Asn Ile Glu Val Val Arg Ser Thr Met Gln Glu Glu Leu Asp Ala
210 215 220
Ala Gln Thr Lys Thr Thr Val Cys Asp Leu Phe Arg Asn Pro Ser Met
225 230 235
Arg Lys Arg Ile Cys Ile Leu Val Phe Leu Arg Lys Lys Ile Ser Arg
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Lys Arg His Lys Asn Asp Cys Tyr Thr Lys Val Thr Lys Phe
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<210> 21
<211> 1759
<212> DNA
<213> Homo sapiens

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cccaccctcg ctcgcgtagc c atg gcg gag ccg tcg gcg gcc act cag tcc 171
Met Ala Glu Pro Ser Ala Ala Thr Gln Ser
1 5 10
cat tcc atc tcc tcg tcg tcc ttc gga gcc gag ccg tcc gcg ccc ggc 219
His Ser Ile Ser Ser Ser Ser Phe Gly Ala Glu Pro Ser Ala Pro Gly
15 20 25
ggc ggc ggg agc cca gga gcc tgc ccc gcc ctg ggg acg aag agc tgc 267

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agc tcc tcc tgt gcg gtg cac gat ctg att ttc tgg aga gat gtg aag      315
Ser Ser Ser Cys Ala Val His Asp Leu Ile Phe Trp Arg Asp Val Lys
        45                  50                  55
aag act ggg ttt gtc ttt ggc acc acg ctg atc atg ctg ctt tcc ctg      363
Lys Thr Gly Phe Val Phe Gly Thr Thr Leu Ile Met Leu Leu Ser Leu
    60                  65                  70
gca gct ttc agt gtc atc agt gtg gtt tct tac ctc atc ctg gct ctt      411
Ala Ala Phe Ser Val Ile Ser Val Val Ser Tyr Leu Ile Leu Ala Leu
75                  80                  85                  90
ctc tct gtc acc atc agc ttc agg atc tac aag tcc gtc atc caa gct      459
Leu Ser Val Thr Ile Ser Phe Arg Ile Tyr Lys Ser Val Ile Gln Ala
                95                  100                 105
gta cag aag tca gaa gaa ggc cat cca ttc aaa gcc tac ctg gac gta      507
Val Gln Lys Ser Glu Glu Gly His Pro Phe Lys Ala Tyr Leu Asp Val
            110                 115                 120
gac att act ctg tcc tca gaa gct ttc cat aat tac atg aat gct gcc      555
Asp Ile Thr Leu Ser Ser Glu Ala Phe His Asn Tyr Met Asn Ala Ala
        125                 130                 135
atg gtg cac atc aac agg gcc ctg aaa ctc att att cgt ctc ttt ctg      603
Met Val His Ile Asn Arg Ala Leu Lys Leu Ile Ile Arg Leu Phe Leu
    140                 145                 150
gta gaa gat ctg gtt gac tcc ttg aag ctg gct gtc ttc atg tgg ctg      651
Val Glu Asp Leu Val Asp Ser Leu Lys Leu Ala Val Phe Met Trp Leu
155                 160                 165                 170
atg acc tat gtt ggt gct gtt ttt aac gga atc acc ctt cta att ctt      699
Met Thr Tyr Val Gly Ala Val Phe Asn Gly Ile Thr Leu Leu Ile Leu
                175                 180                 185
gct gaa ctg ctc att ttc agt gtc ccg att gtc tat gag aag tac aag      747
Ala Glu Leu Leu Ile Phe Ser Val Pro Ile Val Tyr Glu Lys Tyr Lys
            190                 195                 200
acc cag att gat cac tat gtt ggc atc gcc cga gat cag acc aag tca      795
Thr Gln Ile Asp His Tyr Val Gly Ile Ala Arg Asp Gln Thr Lys Ser
        205                 210                 215
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att gtt gaa aag atc caa gca aaa ctc cct gga atc gcc aaa aaa aag 843
Ile Val Glu Lys Ile Gln Ala Lys Leu Pro Gly Ile Ala Lys Lys Lys
220 225 230

gca gaa taagtacatg gaaaccagaa atgcaacagt tactaaaaca ccatttaata g 900
Ala Glu
235

ttataacgtc gttacttgta ctatgaagga aaatactcag tgtcagcttg agcctgcatt 960
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ttcagcacta gtcttactca gctatccatt atagttttgc ccttaagaag tcatgattaa 1260
cttatgaaaa aattatttgg ggacaggagt gtgatacctt ccttggtttt tttttgcagc 1320
cctcaaatcc tatcttcctg ccccacaatg tgagcagcta cccctgatac tccttttctt 1380
taatgattta actatcaact tgataaataa cttataggtg atagtgataa ttcctgattc 1440
caagaatgcc atctgataaa aaagaataga aatggaaagt gggactgaga gggagtcagc 1500
aggcatgctg cggtggcggt cactccctct gccactatcc ccagggaagg aaaggctccg 1560
ccatttggga aagtggtttc tacgtcactg gacaccggtt ctgagcatta gtttgagaac 1620
tcgttcccga atgtgctttc ctccctctcc cctgcccacc tcaagtttaa taaataaggt 1680
tgtacttttc ttactataaa ataaatgtct gtaactgctg tgcactgctg taaacttgtt 1740
agagaaaaaa ataaccctgc 1759

<210> 22
<211> 236
<212> PRT
<213> Homo sapiens

<400> 22

Met Ala Glu Pro Ser Ala Ala Thr Gln Ser
1 5 10
His Ser Ile Ser Ser Ser Ser Phe Gly Ala Glu Pro Ser Ala Pro Gly
15 20 25
Gly Gly Gly Ser Pro Gly Ala Cys Pro Ala Leu Gly Thr Lys Ser Cys
30 35 40
Ser Ser Ser Cys Ala Val His Asp Leu Ile Phe Trp Arg Asp Val Lys

```
        45                  50                  55
Lys Thr Gly Phe Val Phe Gly Thr Thr Leu Ile Met Leu Leu Ser Leu
    60                  65                  70
Ala Ala Phe Ser Val Ile Ser Val Val Ser Tyr Leu Ile Leu Ala Leu
75                  80                  85                  90
Leu Ser Val Thr Ile Ser Phe Arg Ile Tyr Lys Ser Val Ile Gln Ala
                95                  100                 105
Val Gln Lys Ser Glu Glu Gly His Pro Phe Lys Ala Tyr Leu Asp Val
            110                 115                 120
Asp Ile Thr Leu Ser Ser Glu Ala Phe His Asn Tyr Met Asn Ala Ala
        125                 130                 135
Met Val His Ile Asn Arg Ala Leu Lys Leu Ile Ile Arg Leu Phe Leu
    140                 145                 150
Val Glu Asp Leu Val Asp Ser Leu Lys Leu Ala Val Phe Met Trp Leu
155                 160                 165                 170
Met Thr Tyr Val Gly Ala Val Phe Asn Gly Ile Thr Leu Leu Ile Leu
                175                 180                 185
Ala Glu Leu Leu Ile Phe Ser Val Pro Ile Val Tyr Glu Lys Tyr Lys
            190                 195                 200
Thr Gln Ile Asp His Tyr Val Gly Ile Ala Arg Asp Gln Thr Lys Ser
        205                 210                 215
Ile Val Glu Lys Ile Gln Ala Lys Leu Pro Gly Ile Ala Lys Lys Lys
    220                 225                 230
Ala Glu
235
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<210> 23
<211> 1069
<212> DNA
<213> Homo sapiens

<400> 23

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agtggaagac caggcagccc agctgaaggc agtaagctcg gctcacagtc gcaggagagt     60
tctggggtac acgggcaaag gggcttgaga aggcccggag gcgaagccga agagaagcaa    120
ctgtgccccg gagaagagaa gctcgcccat tccagactgg gaaccagctt tcagtgaag     179
```

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atg gca ggg cca gaa ctg ttg ctc gac tcc aac atc cgc ctc tgg gtg      227
Met Ala Gly Pro Glu Leu Leu Leu Asp Ser Asn Ile Arg Leu Trp Val
  1               5                  10                  15
gtc cta ccc atc gtt atc atc act ttc ttc gta ggc atg atc cgc cac      275
Val Leu Pro Ile Val Ile Ile Thr Phe Phe Val Gly Met Ile Arg His
            20                  25                  30
tac gtg tcc atc ctg ctg cag agc gac aag aag ctc acc cag gaa caa      323
Tyr Val Ser Ile Leu Leu Gln Ser Asp Lys Lys Leu Thr Gln Glu Gln
        35                  40                  45
gta tct gac agt caa gtc cta att cga agc aga gtc ctc agg gaa aat      371
Val Ser Asp Ser Gln Val Leu Ile Arg Ser Arg Val Leu Arg Glu Asn
    50                  55                  60
gga aaa tac att ccc aaa cag tct ttc ttg aca cga aaa tat tat ttc      419
Gly Lys Tyr Ile Pro Lys Gln Ser Phe Leu Thr Arg Lys Tyr Tyr Phe
65                  70                  75                  80
aac aac cca gag gat gga ttt ttc aaa aaa act aaa cgg aag gta gtg      467
Asn Asn Pro Glu Asp Gly Phe Phe Lys Lys Thr Lys Arg Lys Val Val
                85                  90                  95
cca cct tct cct atg act gat cct act atg ttg aca gac atg atg aaa      515
Pro Pro Ser Pro Met Thr Asp Pro Thr Met Leu Thr Asp Met Met Lys
            100                 105                 110
ggg aat gta aca aat gtc ctc cct atg att ctt att ggt gga tgg atc      563
Gly Asn Val Thr Asn Val Leu Pro Met Ile Leu Ile Gly Gly Trp Ile
        115                 120                 125
aac atg aca ttc tca ggc ttt gtc aca acc aag gtc cca ttt cca ctg      611
Asn Met Thr Phe Ser Gly Phe Val Thr Thr Lys Val Pro Phe Pro Leu
    130                 135                 140
acc ctc cgt ttt aag cct atg tta cag caa gga atc gag cta ctc aca      659
Thr Leu Arg Phe Lys Pro Met Leu Gln Gln Gly Ile Glu Leu Leu Thr
145                 150                 155                 160
tta gat gca tcc tgg gtg agt tct gca tcc tgg tac ttc ctc aat gta      707
Leu Asp Ala Ser Trp Val Ser Ser Ala Ser Trp Tyr Phe Leu Asn Val
                165                 170                 175
ttt ggg ctt cgg agc att tac tct ctg att ctg ggc caa gat aat gcc      755
Phe Gly Leu Arg Ser Ile Tyr Ser Leu Ile Leu Gly Gln Asp Asn Ala
```

```
                180                 185                 190
gct gac caa tca cga atg atg cag gag cag atg acg gga gca gcc atg      803
Ala Asp Gln Ser Arg Met Met Gln Glu Gln Met Thr Gly Ala Ala Met
        195                 200                 205
gcc atg ccc gca gac aca aac aaa gct ttc aag aca gag tgg gaa gct      851
Ala Met Pro Ala Asp Thr Asn Lys Ala Phe Lys Thr Glu Trp Glu Ala
    210                 215                 220
ttg gag ctg acg gat cac cag tgg gca cta gat gat gtc gaa gaa gag      899
Leu Glu Leu Thr Asp His Gln Trp Ala Leu Asp Asp Val Glu Glu Glu
225                 230                 235                 240
ctc atg gcc aaa gac ctc cac ttc gaa ggc atg ttc aaa aag gaa tta      947
Leu Met Ala Lys Asp Leu His Phe Glu Gly Met Phe Lys Lys Glu Leu
                245                 250                 255
cag acc tct att ttt tgaagaccga gcagggatta gctgtgtcag gaacttgg       1000
Gln Thr Ser Ile Phe
            260
agttgcactt aaccttgtaa ctttgtttgg agctggcacc tcttgaaata aaaaggagga   1060
tgcacgagc                                                          1069
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<210> 24
<211> 261
<212> PRT
<213> Homo sapiens

<400> 24

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Met Ala Gly Pro Glu Leu Leu Leu Asp Ser Asn Ile Arg Leu Trp Val
  1               5                  10                  15
Val Leu Pro Ile Val Ile Ile Thr Phe Phe Val Gly Met Ile Arg His
             20                  25                  30
Tyr Val Ser Ile Leu Leu Gln Ser Asp Lys Lys Leu Thr Gln Glu Gln
         35                  40                  45
Val Ser Asp Ser Gln Val Leu Ile Arg Ser Arg Val Leu Arg Glu Asn
     50                  55                  60
Gly Lys Tyr Ile Pro Lys Gln Ser Phe Leu Thr Arg Lys Tyr Tyr Phe
 65                  70                  75                  80
```

Asn Asn Pro Glu Asp Gly Phe Phe Lys Lys Thr Lys Arg Lys Val Val
85 90 95
Pro Pro Ser Pro Met Thr Asp Pro Thr Met Leu Thr Asp Met Met Lys
100 105 110
Gly Asn Val Thr Asn Val Leu Pro Met Ile Leu Ile Gly Gly Trp Ile
115 120 125
Asn Met Thr Phe Ser Gly Phe Val Thr Thr Lys Val Pro Phe Pro Leu
130 135 140
Thr Leu Arg Phe Lys Pro Met Leu Gln Gln Gly Ile Glu Leu Leu Thr
145 150 155 160
Leu Asp Ala Ser Trp Val Ser Ser Ala Ser Trp Tyr Phe Leu Asn Val
165 170 175
Phe Gly Leu Arg Ser Ile Tyr Ser Leu Ile Leu Gly Gln Asp Asn Ala
180 185 190
Ala Asp Gln Ser Arg Met Met Gln Glu Gln Met Thr Gly Ala Ala Met
195 200 205
Ala Met Pro Ala Asp Thr Asn Lys Ala Phe Lys Thr Glu Trp Glu Ala
210 215 220
Leu Glu Leu Thr Asp His Gln Trp Ala Leu Asp Asp Val Glu Glu Glu
225 230 235 240
Leu Met Ala Lys Asp Leu His Phe Glu Gly Met Phe Lys Lys Glu Leu
245 250 255
Gln Thr Ser Ile Phe
260

<210> 25
<211> 1759
<212> DNA
<213> Homo sapiens

<400> 25
attgtgcagc aggcgggccc ccgcgcggca gggcctgga cccgcgcggc tcccgggg 58
atg gtg agc aag gcg ctg ctg cgc ctc gtg tct gcc gtc aac cgc agg 106
Met Val Ser Lys Ala Leu Leu Arg Leu Val Ser Ala Val Asn Arg Arg
1 5 10 15

```
agg atg aag ctg ctg ctg ggc atc gcc ttg ctg gcc tac gtc gcc tct      154
Arg Met Lys Leu Leu Leu Gly Ile Ala Leu Leu Ala Tyr Val Ala Ser
                20                  25                  30
gtt tgg ggc aac ttc gtt aat atg agc ttt cta ctc aac agg tct atc      202
Val Trp Gly Asn Phe Val Asn Met Ser Phe Leu Leu Asn Arg Ser Ile
            35                  40                  45
cag gaa aat ggt gaa cta aaa att gaa agc aag att gaa gag atg gtt      250
Gln Glu Asn Gly Glu Leu Lys Ile Glu Ser Lys Ile Glu Glu Met Val
        50                  55                  60
gaa cca cta aga gag aaa atc aga gat tta gaa aaa agc ttt acc cag      298
Glu Pro Leu Arg Glu Lys Ile Arg Asp Leu Glu Lys Ser Phe Thr Gln
 65                  70                  75                  80
aaa tac cca cca gta aag ttt tta tca gaa aag gat cgg aaa aga att      346
Lys Tyr Pro Pro Val Lys Phe Leu Ser Glu Lys Asp Arg Lys Arg Ile
                 85                  90                  95
ttg ata aca gga ggc gca ggg ttc gtg ggc tcc cat cta act gac aaa      394
Leu Ile Thr Gly Gly Ala Gly Phe Val Gly Ser His Leu Thr Asp Lys
            100                 105                 110
ctc atg atg gac ggc cac gag gtg acc gtg gtg gac aat ttc ttc acg      442
Leu Met Met Asp Gly His Glu Val Thr Val Val Asp Asn Phe Phe Thr
        115                 120                 125
ggc agg aag aga aac gtg gag cac tgg atc gga cat gag aac ttc gag      490
Gly Arg Lys Arg Asn Val Glu His Trp Ile Gly His Glu Asn Phe Glu
    130                 135                 140
ttg att aac cac gac gtg gtg gag ccc ctc tac atc gag ggc gtg gaa      538
Leu Ile Asn His Asp Val Val Glu Pro Leu Tyr Ile Glu Gly Val Glu
145                 150                 155                 160
gtg cga gtg gcc aga atc ttc aac acc ttt ggg cca cgc atg cac atg      586
Val Arg Val Ala Arg Ile Phe Asn Thr Phe Gly Pro Arg Met His Met
                165                 170                 175
aac gat ggg cga gta gtc agc aac ttc atc ctg cag gcg ctc cag ggg      634
Asn Asp Gly Arg Val Val Ser Asn Phe Ile Leu Gln Ala Leu Gln Gly
            180                 185                 190
gag cca ctc acg gta tac gga tcc ggg tct cag aca agg gcg ttc cag      682
Glu Pro Leu Thr Val Tyr Gly Ser Gly Ser Gln Thr Arg Ala Phe Gln
```

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          195                 200                 205
tac gtc agc gat cta gtg aat ggc ctc gtg gct ctc atg aac agc aac      730
Tyr Val Ser Asp Leu Val Asn Gly Leu Val Ala Leu Met Asn Ser Asn
    210                 215                 220
gtc agc agc ccg gtc aac ctg ggg aac cca gaa gaa cac aca atc cta      778
Val Ser Ser Pro Val Asn Leu Gly Asn Pro Glu Glu His Thr Ile Leu
225                 230                 235                 240
gaa ttt gct cag tta att aaa aac ctt gtt ggt agc gga agt gaa att      826
Glu Phe Ala Gln Leu Ile Lys Asn Leu Val Gly Ser Gly Ser Glu Ile
                245                 250                 255
cag ttt ctc tcc gaa gcc cag gat gac cca cag aaa aga aaa cca gac      874
Gln Phe Leu Ser Glu Ala Gln Asp Asp Pro Gln Lys Arg Lys Pro Asp
            260                 265                 270
atc aaa aaa gca aag ctg atg ctg ggg tgg gag ccc gtg gtc ccg ctg      922
Ile Lys Lys Ala Lys Leu Met Leu Gly Trp Glu Pro Val Val Pro Leu
        275                 280                 285
gag gaa ggt tta aac aaa gca att cac tac ttc cgt aaa gaa ctc gag      970
Glu Glu Gly Leu Asn Lys Ala Ile His Tyr Phe Arg Lys Glu Leu Glu
    290                 295                 300
tac cag gca aat aat cag tac atc ccc aaa cca aag cct gcc aga ata     1018
Tyr Gln Ala Asn Asn Gln Tyr Ile Pro Lys Pro Lys Pro Ala Arg Ile
305                 310                 315                 320
aag aaa gga cgg act cgc cac agc tgaactcctc acttttagga cacaagac      1070
Lys Lys Gly Arg Thr Arg His Ser
                325
taccattgta cacttgatgg gatgtatttt tggctttttt ttgttgtcgt ttaaagaaag   1130
actttaacag gtgtcatgaa gaacaaactg gaatttcatt ctgaagcttg ctttaatgaa   1190
atggatgtgc ctaaaagctc ccctcaaaaa actgcagatt ttgccttgca ctttttgaat   1250
ctctcttttt atgtaaaata gcgtagatgc atctctgcgt attttcaagt ttttttatct   1310
tgctgtgaga gcatatgttg tgactgtcgt tgacagtttt atttactggt ttctttgtga   1370
agctgaaaag gaacattaag cgggacaaaa aatgccgatt ttatttataa aagtgggtac   1430
ttaataaatg agtcgttata ctatgcataa agaaaaatcc tagcagtatt gtcaggtggt   1490
ggtgcgccgg cattgatttt agggcagata aaagaattct gtgtgagagc tttatgtttc   1550
tcttttaatt cagagttttt ccaaggtcta cttttgagtt gcaaacttga ctttgaaata   1610
ttcctgttgg tcatgatcaa ggatatttga aatcactact gtgttttgct gcgtatctgg   1670
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ggcgggggca ggttgggggg cacaaagtta acatattctt ggttaaccat ggttaaatat 1730
gctattttaa taaaatattg aaactcacc 1759

<210> 26
<211> 328
<212> PRT
<213> Homo sapiens

<400> 26

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Met Val Ser Lys Ala Leu Leu Arg Leu Val Ser Ala Val Asn Arg Arg
 1               5                  10                  15
Arg Met Lys Leu Leu Leu Gly Ile Ala Leu Leu Ala Tyr Val Ala Ser
            20                  25                  30
Val Trp Gly Asn Phe Val Asn Met Ser Phe Leu Leu Asn Arg Ser Ile
        35                  40                  45
Gln Glu Asn Gly Glu Leu Lys Ile Glu Ser Lys Ile Glu Glu Met Val
    50                  55                  60
Glu Pro Leu Arg Glu Lys Ile Arg Asp Leu Glu Lys Ser Phe Thr Gln
65                  70                  75                  80
Lys Tyr Pro Pro Val Lys Phe Leu Ser Glu Lys Asp Arg Lys Arg Ile
                85                  90                  95
Leu Ile Thr Gly Gly Ala Gly Phe Val Gly Ser His Leu Thr Asp Lys
            100                 105                 110
Leu Met Met Asp Gly His Glu Val Thr Val Val Asp Asn Phe Phe Thr
        115                 120                 125
Gly Arg Lys Arg Asn Val Glu His Trp Ile Gly His Glu Asn Phe Glu
    130                 135                 140
Leu Ile Asn His Asp Val Val Glu Pro Leu Tyr Ile Glu Gly Val Glu
145                 150                 155                 160
Val Arg Val Ala Arg Ile Phe Asn Thr Phe Gly Pro Arg Met His Met
                165                 170                 175
Asn Asp Gly Arg Val Val Ser Asn Phe Ile Leu Gln Ala Leu Gln Gly
            180                 185                 190
Glu Pro Leu Thr Val Tyr Gly Ser Gly Ser Gln Thr Arg Ala Phe Gln
        195                 200                 205
```

Tyr Val Ser Asp Leu Val Asn Gly Leu Val Ala Leu Met Asn Ser Asn
210 215 220
Val Ser Ser Pro Val Asn Leu Gly Asn Pro Glu Glu His Thr Ile Leu
225 230 235 240
Glu Phe Ala Gln Leu Ile Lys Asn Leu Val Gly Ser Gly Ser Glu Ile
245 250 255
Gln Phe Leu Ser Glu Ala Gln Asp Asp Pro Gln Lys Arg Lys Pro Asp
260 265 270
Ile Lys Lys Ala Lys Leu Met Leu Gly Trp Glu Pro Val Val Pro Leu
275 280 285
Glu Glu Gly Leu Asn Lys Ala Ile His Tyr Phe Arg Lys Glu Leu Glu
290 295 300
Tyr Gln Ala Asn Asn Gln Tyr Ile Pro Lys Pro Lys Pro Ala Arg Ile
305 310 315 320
Lys Lys Gly Arg Thr Arg His Ser
325

<210> 27
<211> 1697
<212> DNA
<213> Homo sapiens

<400> 27
aaaaggatac gggagttcct ccttgctctc gcccctactc tttctggtgt tagatcgagc 60
taccctctaa aagcagttta gagtggtaaa aaaaaaaaaa aacacaccaa acgctcgcag 120
ccacaaaagg g atg aaa ttt ctt ctg gac atc ctc ctg ctt ctc ccg tta 170
Met Lys Phe Leu Leu Asp Ile Leu Leu Leu Leu Pro Leu
1 5 10
ctg atc gtc tgc tcc cta gag tcc ttc gtg aag ctt ttt att cct aag 218
Leu Ile Val Cys Ser Leu Glu Ser Phe Val Lys Leu Phe Ile Pro Lys
15 20 25
agg aga aaa tca gtc acc ggc gaa atc gtg ctg att aca gga gct ggg 266
Arg Arg Lys Ser Val Thr Gly Glu Ile Val Leu Ile Thr Gly Ala Gly
30 35 40 45
cat gga att ggg aga ctg act gcc tat gaa ttt gct aaa ctt aaa agc 314

```
His Gly Ile Gly Arg Leu Thr Ala Tyr Glu Phe Ala Lys Leu Lys Ser
                50                  55                  60
aag ctg gtt ctc tgg gat ata aat aag cat gga ctg gag gaa aca gct     362
Lys Leu Val Leu Trp Asp Ile Asn Lys His Gly Leu Glu Glu Thr Ala
 65                  70                  75
gcc aaa tgc aag gga ctg ggt gcc aag gtt cat acc ttt gtg gta gac     410
Ala Lys Cys Lys Gly Leu Gly Ala Lys Val His Thr Phe Val Val Asp
        80                  85                  90
tgc agc aac cga gaa gat att tac agc tct gca aag aag gtg aag gca     458
Cys Ser Asn Arg Glu Asp Ile Tyr Ser Ser Ala Lys Lys Val Lys Ala
    95                  100                 105
gaa att gga gat gtt agt att tta gta aat aat gct ggt gta gtc tat     506
Glu Ile Gly Asp Val Ser Ile Leu Val Asn Asn Ala Gly Val Val Tyr
110                 115                 120                 125
aca tca gat ttg ttt gct aca caa gat cct cag att gaa aag act ttt     554
Thr Ser Asp Leu Phe Ala Thr Gln Asp Pro Gln Ile Glu Lys Thr Phe
                130                 135                 140
gaa gtt aat gta ctt gca cat ttc tgg act aca aag gca ttt ctt cct     602
Glu Val Asn Val Leu Ala His Phe Trp Thr Thr Lys Ala Phe Leu Pro
            145                 150                 155
gca atg acg aag aat aac cat ggc cat att gtc act gtg gct tcg gca     650
Ala Met Thr Lys Asn Asn His Gly His Ile Val Thr Val Ala Ser Ala
        160                 165                 170
gct gga cat gtc tcg gtc ccc ttc tta ctg gct tac tgt tca agc aag     698
Ala Gly His Val Ser Val Pro Phe Leu Leu Ala Tyr Cys Ser Ser Lys
    175                 180                 185
ttt gct gct gtt gga ttt cat aaa act ttg aca gat gaa ctg gct gcc     746
Phe Ala Ala Val Gly Phe His Lys Thr Leu Thr Asp Glu Leu Ala Ala
190                 195                 200                 205
tta caa ata act gga gtc aaa aca aca tgt ctg tgt cct aat ttc gta     794
Leu Gln Ile Thr Gly Val Lys Thr Thr Cys Leu Cys Pro Asn Phe Val
                210                 215                 220
aac act ggc ttc atc aaa aat cca agt aca agt ttg gga ccc act ctg     842
Asn Thr Gly Phe Ile Lys Asn Pro Ser Thr Ser Leu Gly Pro Thr Leu
            225                 230                 235
```

gaa cct gag gaa gtg gta aac agg ctg atg cat ggg att ctg act gag 890
Glu Pro Glu Glu Val Val Asn Arg Leu Met His Gly Ile Leu Thr Glu
240 245 250
cag aag atg att ttt att cca tct tct ata gct ttt tta aca aca ttg 938
Gln Lys Met Ile Phe Ile Pro Ser Ser Ile Ala Phe Leu Thr Thr Leu
255 260 265
gaa agg atc ctt cct gag cgt ttc ctg gca gtt tta aaa cga aaa atc 986
Glu Arg Ile Leu Pro Glu Arg Phe Leu Ala Val Leu Lys Arg Lys Ile
270 275 280 285
agt gtt aag ttt gat gca gtt att gga tat aaa atg aaa gcg caa 1031
Ser Val Lys Phe Asp Ala Val Ile Gly Tyr Lys Met Lys Ala Gln
290 295 300
taagcacct agttttctga aaactgattt accaggttta ggttgatgtc atctaatagt 1090
gccagaattt taatgtttga acttctgttt tttctaatta tccccatttc ttcaatatca 1150
tttttgaggc tttggcagtc ttcatttact accacttgtt ctttagccaa aagctgatta 1210
catatgatat aaacagagaa ataccttag aggtgacttt aaggaaaatg aagaaaaaga 1270
accaaaatga ctttattaaa ataatttcca agattatttg tggctcacct gaaggctttg 1330
caaaatttgt accataaccg tttatttaac atatatttt atttttgatt gcacttaaat 1390
tttgtataat ttgtgtttct ttttctgttc tacataaaat cagaaacttc aagctctcta 1450
aataaaatga aggactatat ctagtggtat ttcacaatga atatcatgaa ctctcaatgg 1510
gtaggtttca tcctacccat tgccactctg tttcctgaga gatacctcac attccaatgc 1570
caaacatttc tgcacaggga agctagaggt ggatacacgt gttgcaagta taaaagcatc 1630
actgggattt aaggagaatt gagagaatgt acccacaaat ggcagcaata ataaatggat 1690
cacactt 1697

<210> 28
<211> 300
<212> PRT
<213> Homo sapiens

<400> 28

Met Lys Phe Leu Leu Asp Ile Leu Leu Leu Leu Pro Leu
1 5 10
Leu Ile Val Cys Ser Leu Glu Ser Phe Val Lys Leu Phe Ile Pro Lys
15 20 25

Arg Arg Lys Ser Val Thr Gly Glu Ile Val Leu Ile Thr Gly Ala Gly
30 35 40 45
His Gly Ile Gly Arg Leu Thr Ala Tyr Glu Phe Ala Lys Leu Lys Ser
50 55 60
Lys Leu Val Leu Trp Asp Ile Asn Lys His Gly Leu Glu Glu Thr Ala
65 70 75
Ala Lys Cys Lys Gly Leu Gly Ala Lys Val His Thr Phe Val Val Asp
80 85 90
Cys Ser Asn Arg Glu Asp Ile Tyr Ser Ser Ala Lys Lys Val Lys Ala
95 100 105
Glu Ile Gly Asp Val Ser Ile Leu Val Asn Asn Ala Gly Val Val Tyr
110 115 120 125
Thr Ser Asp Leu Phe Ala Thr Gln Asp Pro Gln Ile Glu Lys Thr Phe
130 135 140
Glu Val Asn Val Leu Ala His Phe Trp Thr Thr Lys Ala Phe Leu Pro
145 150 155
Ala Met Thr Lys Asn Asn His Gly His Ile Val Thr Val Ala Ser Ala
160 165 170
Ala Gly His Val Ser Val Pro Phe Leu Leu Ala Tyr Cys Ser Ser Lys
175 180 185
Phe Ala Ala Val Gly Phe His Lys Thr Leu Thr Asp Glu Leu Ala Ala
190 195 200 205
Leu Gln Ile Thr Gly Val Lys Thr Thr Cys Leu Cys Pro Asn Phe Val
210 215 220
Asn Thr Gly Phe Ile Lys Asn Pro Ser Thr Ser Leu Gly Pro Thr Leu
225 230 235
Glu Pro Glu Glu Val Val Asn Arg Leu Met His Gly Ile Leu Thr Glu
240 245 250
Gln Lys Met Ile Phe Ile Pro Ser Ser Ile Ala Phe Leu Thr Thr Leu
255 260 265
Glu Arg Ile Leu Pro Glu Arg Phe Leu Ala Val Leu Lys Arg Lys Ile
270 275 280 285
Ser Val Lys Phe Asp Ala Val Ile Gly Tyr Lys Met Lys Ala Gln
290 295 300

<210> 29
<211> 814
<212> DNA
<213> Homo sapiens

<400> 29

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agaatcccgg acagccctgc tccctgcagc caggtgtagt ttcgggagcc actggggcca      60
aagtgagagt ccagcggtct tccagcgctt gggccacggc ggcggccctg ggagcagagg     120
tggagcgacc ccattacgct aaag atg aaa ggc tgg ggt tgg ctg gcc ctg         171
                           Met Lys Gly Trp Gly Trp Leu Ala Leu
                             1               5
ctt ctg ggg gcc ctg ctg gga acc gcc tgg gct cgg agg agc cag gat       219
Leu Leu Gly Ala Leu Leu Gly Thr Ala Trp Ala Arg Arg Ser Gln Asp
 10                  15                  20                  25
ctc cac tgt gga gca tgc agg gct ctg gtg gat gaa cta gaa tgg gaa       267
Leu His Cys Gly Ala Cys Arg Ala Leu Val Asp Glu Leu Glu Trp Glu
                30                  35                  40
att gcc cag gtg gac ccc aag aag acc att cag atg gga tct ttc cgg       315
Ile Ala Gln Val Asp Pro Lys Lys Thr Ile Gln Met Gly Ser Phe Arg
            45                  50                  55
atc aat cca gat ggc agc cag tca gtg gtg gag gtg cct tat gcc cgc       363
Ile Asn Pro Asp Gly Ser Gln Ser Val Val Glu Val Pro Tyr Ala Arg
        60                  65                  70
tca gag gcc cac ctc aca gag ctg ctg gag gag ata tgt gac cgg atg       411
Ser Glu Ala His Leu Thr Glu Leu Leu Glu Glu Ile Cys Asp Arg Met
    75                  80                  85
aag gag tat ggg gaa cag att gat cct tcc acc cat cgc aag aac tac       459
Lys Glu Tyr Gly Glu Gln Ile Asp Pro Ser Thr His Arg Lys Asn Tyr
 90                  95                 100                 105
gta cgt gta gtg ggc cgg aat gga gaa tcc agt gaa ctg gac cta caa       507
Val Arg Val Val Gly Arg Asn Gly Glu Ser Ser Glu Leu Asp Leu Gln
                110                 115                 120
ggc atc cga atc gac tca gat att agc ggc acc ctc aag ttt gcg tgt       555
Gly Ile Arg Ile Asp Ser Asp Ile Ser Gly Thr Leu Lys Phe Ala Cys
            125                 130                 135
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gag agc att gtg gag gaa tac gag gat gaa ctc att gaa ttc ttt tcc 603
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Arg Glu Ala Asp Asn Val Lys Asp Lys Leu Cys Ser Lys Arg Thr Asp
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Asn Phe Asp Val Arg Pro Gln Ser Gly Ala Asn Gly Leu Pro Lys His
            30                  35                  40
tcc tac tgg ttg gac ctc tgg ctt ttc atc ctt ttc gat gtg gtg gtg      196
Ser Tyr Trp Leu Asp Leu Trp Leu Phe Ile Leu Phe Asp Val Val Val
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Phe Leu Phe Val Tyr Phe Leu Pro
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tctcaataga agaatttcac atctccaagg gacccttcct ttcattttac actttgttac    370
taatttgcag aactctatta attgggtagg atttcaccca ttcctagcta agtttcttaaa    430
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                                                               1
gcg tct cga gca ggc ccg cga gcg gcc ggc acc gac ggc agc gac ttt       164
Ala Ser Arg Ala Gly Pro Arg Ala Ala Gly Thr Asp Gly Ser Asp Phe
            5                  10                  15
cag cac cgg gag cgc gtc gcc atg cac tac cag atg agt gtg acc ctc       212
Gln His Arg Glu Arg Val Ala Met His Tyr Gln Met Ser Val Thr Leu
        20                  25                  30
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Lys Tyr Glu Ile Lys Lys Leu Ile Tyr Val His Leu Val Ile Trp Leu
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    att ttg ccc tct ctc ttg ggc ctt ctc tcc ttt ccc cgc aac aac att      404
    Ile Leu Pro Ser Leu Leu Gly Leu Leu Ser Phe Pro Arg Asn Asn Ile
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    agc tac ctg gtg ctc tcc atg atc agc atg gga ctc ttt tcc atc gct      452
    Ser Tyr Leu Val Leu Ser Met Ile Ser Met Gly Leu Phe Ser Ile Ala
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    Pro Leu Ile Tyr Gly Ser Met Glu Met Phe Pro Ala Ala Gln Gln Leu
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    tac cgc cat ggc aag gcc tac cgt ttc ctc ttt ggt ttt tct gcc gtt      548
    Tyr Arg His Gly Lys Ala Tyr Arg Phe Leu Phe Gly Phe Ser Ala Val
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    Gln Leu Tyr Tyr Ser Lys Lys Leu Leu Asp Ser Trp Phe Thr Ser Thr
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    Gln Glu Lys Lys His Lys
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Asp Gln Val Ala Met Pro Tyr Gln Trp Glu Tyr Pro Tyr Leu Leu Ser
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Ser Tyr Leu Val Leu Ser Met Ile Ser Met Gly Leu Phe Ser Ile Ala
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Pro Leu Ile Tyr Gly Ser Met Glu Met Phe Pro Ala Ala Gln Gln Leu
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Tyr Arg His Gly Lys Ala Tyr Arg Phe Leu Phe Gly Phe Ser Ala Val
130 135 140 145
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Ala Ala Leu Ala Glu Ser Ala Gly Pro Leu His Gln Leu Ala Gln Ser
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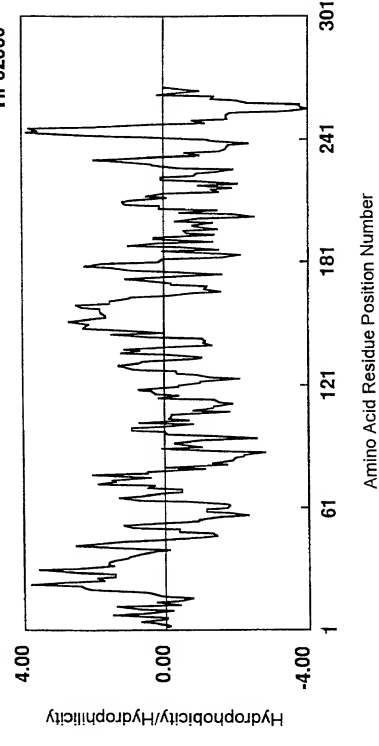
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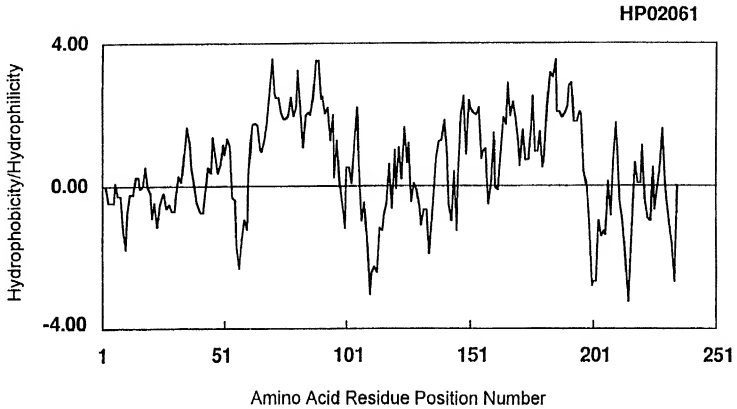
Fig. 1



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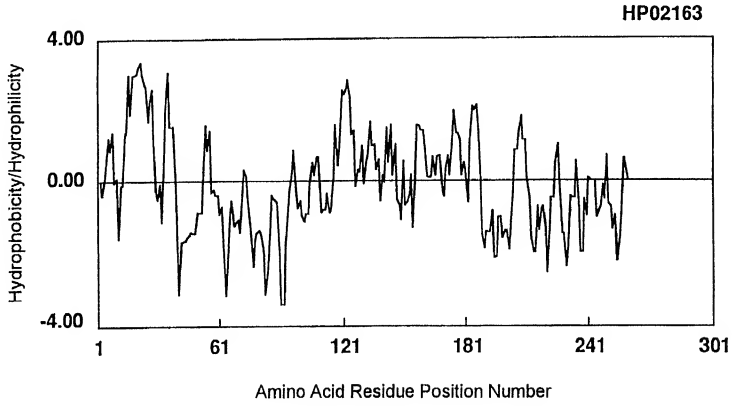

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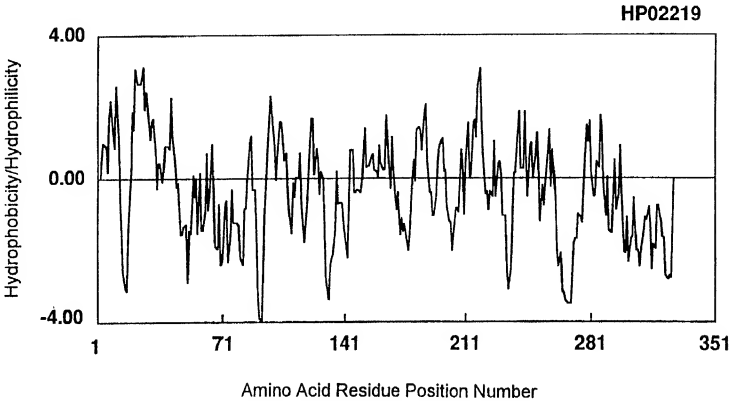

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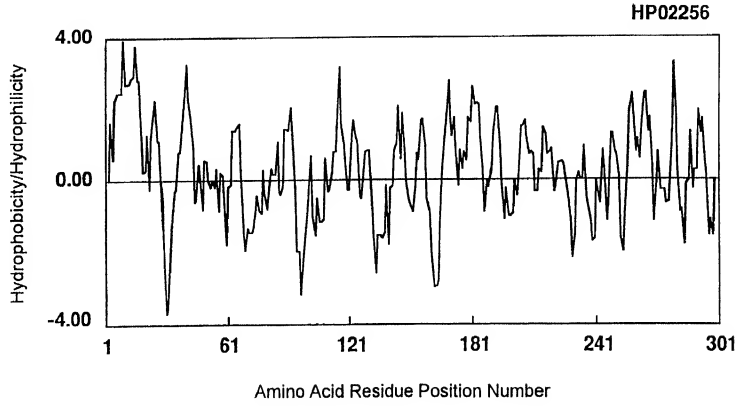
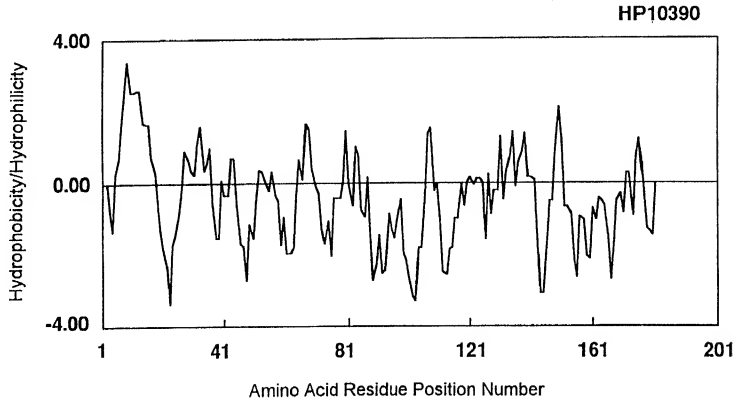


Fig. 5



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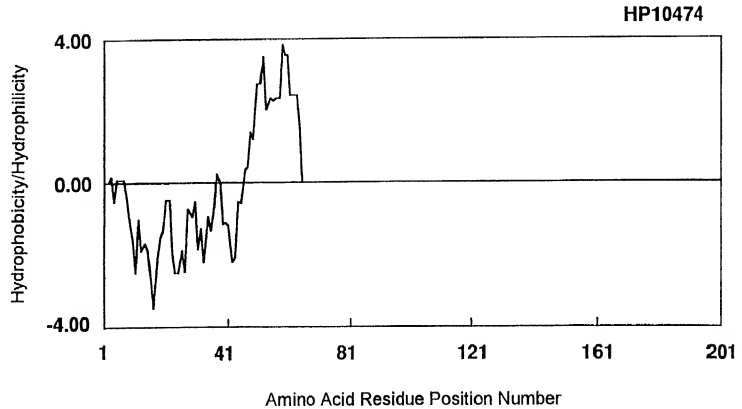

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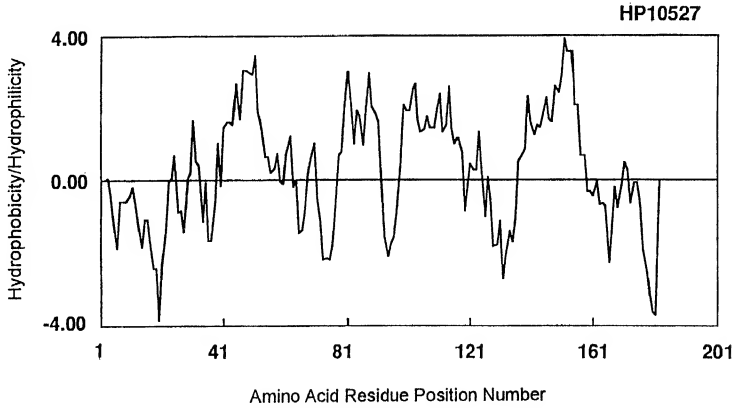

Fig. 8

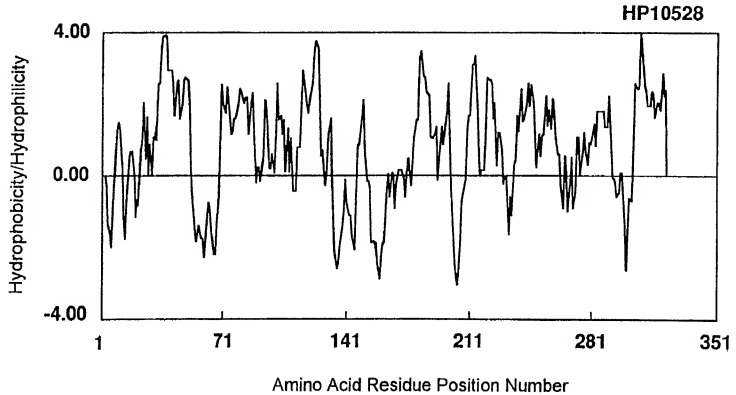

Fig. 9

#20

DECLARATION, PETITION AND POWER OF ATTORNEY FOR PATENT APPLICATION

(Check one):

☐ Declaration Submitted with Initial Filing

☒ Declaration Submitted after Initial Filing

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

HUMAN PROTEINS HAVING TRANSMEMBRANE DOMAINS AND DNAS ENCODING THESE PROTEINS

the specification of which (check one):

☐ is attached hereto.

OR

☒ was filed on **October 27, 2000** as U.S. National Application Serial No. 09/674,235 **(U.S. National Filing of PCT/JP99/02226 filed on April 27, 1999).**

☐ and was amended by PCT Article 19 Amendment on _____ (if applicable),

☐ and was amended by PCT Article 34 Amendment on _____ (if applicable).

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby state that I have reviewed and understood the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

PRIORITY CLAIM

(Check one):

☐ no such applications have been filed.

☒ such applications have been filed as follows

1) FOREIGN PRIORITY CLAIM: I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate or §365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (dd/mm/yyyy)	Priority Not Claimed	Certified Copy Attached Yes	Certified Copy Attached No
10/119395	**JP**	**28 April 1998 (28.04.98)**	☐	☐	☒
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority sheet attached hereto.

2) PROVISIONAL PRIORITY CLAIM: I hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below.

Provisional Application Number(s)	Filing Date (dd/mm/yyyy)

☐ Additional provisional application numbers are listed on a supplemental priority sheet attached hereto.

3) U.S./PCT PRIORITY CLAIM: I hereby claim the benefit under Title 35, United States Code, §120 of any United States application or §365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application Number	PCT Parent Number	Parent Filing Date (dd/mm/yyyy)	Parent Patent Number *(if applicable)*
	PCT/JP99/02226	**27 April 1999 (27.04.99)**	

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority sheet attached hereto.

POWER OF ATTORNEY:

As a named inventor, I hereby appoint the following attorneys and/or agents to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

James E. Cockfield	Reg. No. 19,162	Nicholas P. Triano III	Reg. No. 36,397
Thomas V. Smurzynski	Reg. No. 24,798	Peter C. Lauro	Reg. No. 32,360
Ralph A. Loren	Reg. No. 29,325	DeAnn F. Smith	Reg. No. 36,683
Giulio A. DeConti, Jr.	Reg. No. 31,503	William D. DeVaul	Reg. No. 42,483
Ann Lamport Hammitte	Reg. No. 34,858	David J. Rikkers	Reg. No. 43,882
Elizabeth A. Hanley	Reg. No. 33,505	Chi Suk Kim	Reg. No. 42,728
Amy E. Mandragouras	Reg. No. 36,207	Maria Laccotripe Zacharakis	Limited Recognition
Anthony A. Laurentano	Reg. No. 38,220		Under 37 C.F.R. § 10.9(b)
Jane E. Remillard	Reg. No. 38,872	Debra J. Milasincic	Reg. No. 46,931
Jeremiah Lynch	Reg. No. 17,425	David R. Burns	Reg. No. 46,590
Kevin J. Canning	Reg. No. 35,470	Sean D. Detweiler	Reg. No. 42,482
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Wherefore I petition that letters patent be granted to me for the invention or discovery described and claimed in the attached specification and claims, and hereby subscribe my name to said specification and claims and to the foregoing declaration, power of attorney, and this petition.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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